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**UTILITY
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(Only for new nonprovisional applications under 37 CFR 1.53(b))

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First Named Inventor or Application Identifier

Michal Eisenbach-Schwartz

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APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents.

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 - Cross Reference to Related Applications
 - Statement Regarding Fed sponsored R&D
 - Reference to Microfiche Appendix
 - Background of the Invention
 - Brief Summary of the Invention
 - Brief Description of the Drawings (if filed)
 - Detailed Description of the Invention (including drawings, if filed)
 - Claim(s)
 - Abstract of the Disclosure
3. ☒ Drawing(s) (35 USC 113) (17 Figures/25 Sheets) [Total Sheets 25]
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 - a. ☐ Newly executed (original or copy)
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(for continuation/divisional with Box 17 completed)
[Note Box 5 below]
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ATTORNEY DOCKET NO. 5763-029Date December 22, 1998

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Applicant(s): Michal Eisenbach-Schwartz, Irun R. Cohen, Gila Moalem, Pierre Beserman, and Alon Monsonego Executed on: Inventors unavailable at time of execution

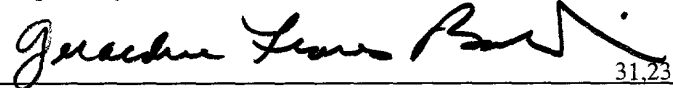
Title of Invention: ACTIVATED T-CELLS, NERVOUS SYSTEM-SPECIFIC ANTIGENS AND THEIR USES**PATENT APPLICATION FEE VALUE**

TYPE	NO. FILED	LESS	EXTRA	EXTRA RATE	FEE
Total Claims	30	-20	10	\$18.00 each	180.00
Independent	2	-3	0	\$78.00 each	0.00
Minimum Fee					760.00
Multiple Dependency Fee If Applicable (\$260.00)					260.00
Total					1,200.00
50% Reduction for Independent Inventor, Nonprofit Organization or Small Business Concern (a verified statement as to the applicant's status is attached)					-
Total Filing Fee					\$ 1,200.00

- ☒ Priority of application no. IL 124550 filed on May 19, 1998 in Israel is claimed under 35 U.S.C. § 119.
☐ The certified copy of the priority application has been filed in application no. filed.
☐ Amend the specification by inserting before the first line the following sentence; This is a continuation-in-part of application no. filed.

Please charge the required fee to Pennie & Edmonds LLP Deposit Account No. 16-1150. A copy of this sheet is enclosed.

Respectfully submitted,



Geraldine F. Baldwin
PENNIE & EDMONDS LLP

31,232
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Enclosure

This form is not for use with continuation, divisional, re-issue, design or plant patent applications.

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**ACTIVATED T-CELLS, NERVOUS SYSTEM-SPECIFIC
ANTIGENS AND THEIR USES**

5 The present application is a continuation-in-part of
PCT/US98/14715, filed July 21, 1998. The present application
claims priority benefit under 35 U.S.C. § 119 of copending
Israeli patent application IL 124550, filed May 19, 1998, the
disclosure of which is incorporated herein by reference in its
entirety and priority benefit under 35 U.S.C. § 120 of
10 PCT/US98/14715, filed July 21, 1998.

1. FIELD OF THE INVENTION

15 The present invention relates to compositions and methods
for the promotion of nerve regeneration or prevention or
inhibition of axon degeneration to ameliorate the effects of
injury or disease of the nervous system (NS). In certain
embodiments, activated antiseif T-cells, a NS-specific antigen
or peptide derived therefrom or a nucleotide sequence encoding
a NS-specific antigen or peptide derived therefrom are/is used
20 to promote nerve regeneration or to prevent or inhibit axonal
degeneration caused by injury or disease of nerves within the
CNS or PNS of a human subject. The compositions of the present
invention may be administered alone or may be optionally
administered in any desired combination.

25 **2. BACKGROUND OF THE INVENTION**

The nervous system comprises the central and the
peripheral nervous system (PNS). The central nervous system
(CNS) is composed of the brain and spinal cord; the PNS
consists of all the other neural elements, namely the nerves
30 and ganglia outside the brain and spinal cord.

Damage to the NS may result from a traumatic injury, such
as penetrating trauma or blunt trauma, or a disease or

disorder, including but not limited to Alzheimer's disease, Parkinson's disease, multiple sclerosis, Huntington's disease, amyotrophic lateral sclerosis (ALS), Diabetic neuropathy, senile dementia, and ischemia.

5 Maintenance of CNS integrity is a complex 'balancing act' in which compromises are struck with the immune system. In most tissues, the immune system plays an essential part in protection, repair and healing. In the CNS, because of its unique immune privilege, immunological reactions are relatively
10 limited (Streilein, J.W., 1993, *Curr. Opin. Immunol.* 5:428-432; Streilein, J.W., 1993, *Science*, 270:1158-1159). A growing body of evidence indicates that the failure of the mammalian CNS to achieve functional recovery after injury is a reflection of an ineffective 'dialog' between the damaged tissue and the immune
15 system. For example, the restricted communication between the CNS and blood-borne macrophages affects the capacity of axotomized axons to regrow; transplantation of activated macrophages can promote CNS regrowth (Lazarov Spiegler, O., et al., 1996, *FASEB J.*, 10:1296-1302; Rapalino, O. et al., 1998,
20 *Nature Med.* 4:814-821).

Activated T cells have been shown to enter the CNS parenchyma, irrespective of their antigen specificity, but only T cells capable of reacting with a CNS antigen seem to persist there (Hickey, W.F., et al., 1991, *J. Neurosci. Res.* 28:254-
25 260). T cells reactive to antigens of CNS white matter, such as myelin basic protein (MBP), can induce the paralytic disease experimental autoimmune encephalomyelitis (EAE) within several days of their inoculation into naive recipient rats (Ben Nun, A., et al., 1981, *Eur. J. Immunol.* 11:195-199). Anti-MBP T
30 cells may also be involved in the human disease multiple sclerosis (Ota, K., et al., 1990, *Nature* 346:183-187; Martin, R., 1997, *J. Neural Transm. Suppl.* 49:53-67). However, despite

their pathogenic potential, anti-MBP T-cell clones are present in the immune systems of healthy subjects (Burns, J., et al., 1983, *Cell. Immunol.* 81:435-440; Pette, M., et al., 1990, *Proc. Natl. Acad. Sci. USA* 87:7968-7972; Martin, R., et al., 1990, *J. Immunol.* 145:540-548; Schiuesener, H.J., et al., 1985, *J. Immunol.* 135:3128-3133). Activated T cells, which normally patrol the intact CNS, transiently accumulate at sites of CNS white matter lesions (Hirschberg, D.L., et al., 1998, *J. Neuroimmunol.* 89:88-96).

10 A catastrophic consequence of CNS injury is that the primary damage is often compounded by the gradual secondary loss of adjacent neurons that apparently were undamaged, or only marginally damaged, by the initial injury (Faden, A.I., et al., 1992, *Trends Pharmacol. Sci.* 13:29-35; Faden, A.I., 1993, *Crit. Rev. Neurobiol.* 7:175-186; McIntosh, T.K., 1993, *J. Neurotrauma* 10:215-261). The primary lesion causes changes in extracellular ion concentrations, elevation of amounts of free radicals, release of neurotransmitters, depletion of growth factors, and local inflammation. These changes trigger a

15 cascade of destructive events in the adjacent neurons that initially escaped the primary injury (Lynch, D.R., et al., 1994, *Curr. Opin. Neurol.* 7:510-516; Bazan, N.G., et al., 1995, *J. Neurotrauma* 12:791-814; Wu, D., et al., 1994, *J. Neurochem.* 62:37-44). This secondary damage is mediated by activation of

20 voltage-dependent or agonist-gated channels, ion leaks, activation of calcium-dependent enzymes such as proteases, lipases and nucleases, mitochondrial dysfunction and energy depletion, culminating in neuronal cell death (Yoshina, A., et al., 1991, *Brain Res.* 561:106-119; Hovda, D.A., et al., 1991, *Brain Res.* 567:1-10; Zivin, J.A., et al., 1991, *Sci. Am.* 265:56-63; Yoles, E., et al., 1992, *Invest. Ophthalmol. Vis. Sci.* 33:3586-3591). The widespread loss of neurons beyond the

30

loss caused directly by the primary injury has been called
'secondary degeneration'.

Another tragic consequence of CNS injury is that neurons
in mammalian CNS do not undergo spontaneous regeneration
5 following an injury. Thus, a CNS injury causes permanent
impairment of motor and sensory functions.

Citation or identification of any reference in this
section or any other part of this specification shall not be
construed as an admission that such reference is available as
10 prior art to the present invention.

3. SUMMARY OF THE INVENTION

The present invention is directed to methods and
compositions for the promotion of nerve regeneration or
15 prevention or inhibition of axonal degeneration to ameliorate
the effects of injury or disease of the nervous system (NS).
The present invention is based, in part, on the Applicants'
unexpected discovery, that non-recombinant antiself T-cells
that recognize an antigen of the NS or a peptide derived
20 therefrom promote nerve regeneration or confer neuroprotection.
As used herein, "neuroprotection" refers to the prevention or
inhibition of degenerative effects of injury or disease in the
NS. Until recently, it was thought that the immune system
excluded immune cells from participating in nervous system
25 repair. It was quite surprising to discover that non-
recombinant NS-specific antiself activated T-cells can be used
to promote nerve regeneration or to protect nervous system
tissue from secondary degeneration which may follow damage
caused by injury or disease of the CNS or PNS, in particular,
30 a lesion other than a neoplasm or an autoimmune disease
affecting the NS.

"Activated T-cell" as used herein includes (i) T-cells

that have been activated by exposure to a cognate antigen or peptide derived therefrom or derivative thereof and (ii) progeny of such activated T-cells. As used herein, a cognate antigen is an antigen that is specifically recognized by the T-cell antigen receptor of a T-cell that has been previously exposed to the antigen.

In an embodiment, the present invention provides pharmaceutical compositions comprising a therapeutically effective amount of non-recombinant, NS-specific antiself activated T-cells and methods of use of such compositions for promotion of nerve regeneration or for prevention or inhibition of axonal degeneration in the CNS or PNS in which the amount is effective to ameliorate the effects of an injury or disease of the NS. "NS-specific antiself activated T-cell" as used herein refers to an activated T-cell having specificity for an antigen of the NS or a peptide derived therefrom. Preferably, the NS-specific antiself activated T cells are used to promote nerve regeneration or to prevent or inhibit the effects of disease in which the disease is not an autoimmune disease or a neoplasm.

The present invention also provides pharmaceutical compositions comprising a therapeutically effective amount of a NS-specific antigen or peptide derived therefrom or derivative thereof and methods of use of such compositions for promotion of nerve regeneration or for prevention or inhibition of axonal degeneration in the CNS or PNS in which the amount is effective to activate T-cells *in vivo* or *in vitro* wherein the activated T-cells inhibit or ameliorate the effects of an injury or disease of the NS. "NS-specific antigen" as used herein refers to an antigen that specifically activates T-cells such that following activation the activated T-cells accumulate at a site of injury or disease in the NS. Preferably, the NS-specific antigen is used to promote regeneration or to prevent

or inhibit the effects of disease in which the disease is not an autoimmune disease or a neoplasm. In an embodiment, the peptide derived from a NS-specific antigen is a "cryptic epitope" of the antigen. A cryptic epitope activates specific
5 T cells after an animal is immunized with the particular peptide, but not with the whole antigen. In another embodiment, the peptide derived from a NS-specific antigen is an immunogenic epitope of the antigen. "Derivatives" of NS-specific antigens or peptides derived therefrom as used herein
10 refers to analogs or chemical derivatives of such antigens or peptides as described below, see Section 5.2.

The present invention also provides pharmaceutical compositions comprising a therapeutically effective amount of a nucleotide sequence encoding a NS-specific antigen or peptide
15 derived therefrom or derivative thereof and methods of use of such compositions for promotion of nerve regeneration or for prevention or inhibition of axonal degeneration in the CNS or PNS in which the amount is effective to ameliorate the effects of an injury or disease of the NS.

20 In the practice of the invention, therapy for amelioration of effects of injury or disease comprising administration of NS-specific antiseif activated T-cells may optionally be in combination with a NS-specific antigen or peptide derived therefrom or derivative thereof or a nucleotide sequence
25 encoding a NS-specific antigen or peptide derived therefrom.

4. BRIEF DESCRIPTION OF THE FIGURES

Fig. 1 shows T-cell presence in injured optic nerve 1 week after injury. Adult Lewis rats were injected with activated T
30 cells of the anti-MBP (T_{MBP}), anti-OVA (T_{OVA}), or anti-p277 (T_{p277}) lines, or with PBS, immediately after unilateral crush injury of the optic nerve. Seven days later, both the injured and

uninjured optic nerves were removed, cryosectioned and analyzed immunohistochemically for the presence of immunolabeled T cells. T cells were counted at the site of injury and at randomly selected areas in the uninjured optic nerves. The
5 histogram shows the mean number of T cells per $\text{mm}^2 \pm \text{s.e.m.}$, counted in two to three sections of each nerve. Each group contained three to four rats. The number of T cells was considerably higher in injured nerves of rats injected with anti-MBP, anti-OVA or anti-p277 T cells; statistical analysis
10 (one-way ANOVA) showed significant differences between T cell numbers in injured optic nerves of rats injected with anti-MBP, anti-OVA, or anti-p277 T cells and in injured optic nerves of rats injected with PBS ($P < 0.001$); and between injured optic nerves and uninjured optic nerves of rats injected with anti-
15 MBP, anti-OVA, or anti-p277 T cells ($P < 0.001$).

Fig. 2 illustrates that T cells specific to MBP, but not to OVA or p277 or hsp60, protect neurons from secondary degeneration. Immediately after optic nerve injury, rats were injected with anti-MBP, anti-OVA or anti-p277 T cells, or with
20 PBS. The neurotracer dye 4-Di-10-Asp was applied to optic nerves distal to the site of the injury, immediately after injury (for assessment of primary damage) or 2 weeks later (for assessment of secondary degeneration). Five days after dye application, the retinas were excised and flat-mounted.
25 Labeled retinal ganglion cells (RGCs) from three to five randomly selected fields in each retina (all located at approximately the same distance from the optic disk) were counted by fluorescence microscopy. RGC survival in each group of injured nerves was expressed as the percentage of the total
30 number of neurons spared after the primary injury (42% of axons remained undamaged after the primary injury). The neuroprotective effect of anti-MBP T cells compared with that

of PBS was significant ($P < 0.001$, one-way ANOVA). Anti-OVA T cells or anti-p277 T cells did not differ significantly from PBS in their effects on the protection of neurons that had escaped primary injury ($P > 0.05$, one-way ANOVA). The results
5 are a summary of five experiments. Each group contained five to ten rats.

Figs. 3(A-C) present photomicrographs of retrogradely labeled retinas of injured optic nerves of rats. Immediately after unilateral crush injury of their optic nerves, rats were
10 injected with PBS (Fig. 3A) or with activated anti-p277 T cells (Fig. 3B) or activated anti-MBP T cells (Fig. 3C). Two weeks later, the neurotracer dye 4-Di-10-Asp was applied to the optic nerves, distal to the site of injury. After 5 days, the retinas were excised and flat-mounted. Labeled (surviving)
15 RGCs, located at approximately the same distance from the optic disk in each retina, were photographed.

Figs. 4(A-B) show that clinical severity of EAE is not influenced by an optic nerve crush injury. For the results presented in Fig. 4A, Lewis rats, either uninjured (dash line)
20 or immediately after optic nerve crush injury (solid line), were injected with activated anti-MBP T cells. EAE was evaluated according to a neurological paralysis scale. [Data points represent \pm s.e.m.] These results represent a summary of three experiments. Each group contained five to nine rats.
25 Fig. 4B shows that the number of RGCs in the uninjured optic nerve is not influenced by injection of anti-MBP T cells. Two weeks after the injection of anti-MBP T cells or PBS, 4-Di-10-Asp was applied to the optic nerves. After 5 days the retinas were excised and flat-mounted. Labeled RGCs from five fields
30 (located at approximately the same distance from the optic disk) in each retina were counted and the average number per mm^2 was calculated. There was no difference between the numbers of

labeled RGCs in rats injected with anti-MBP T cells (T_{MBP}) and in PBS-injected control rats.

Fig. 5 shows that T cells specific to p51-70 of MBP protect neurons from secondary degeneration. Immediately after optic nerve injury, rats were injected with anti-MBP T cells, anti-p51-70 T cells, or PBS. The neurotracer dye 4-Di-10-Asp was applied to optic nerves distal to the site of the injury, immediately after injury (for assessment of primary damage) or 2 weeks later (for assessment of secondary degeneration). Five days after dye application, the retinas were excised and flat-mounted. Labeled retinal ganglion cells (RGCs) from three to five randomly selected fields in each retina (all located at approximately the same distance from the optic disk) were counted by fluorescence microscopy. RGC survival in each group of injured nerves was expressed as the percentage of the total number of neurons spared after primary injury. Compared with that of PBS treatment, the neuroprotective effects of anti-MBP and anti-p51-70 T cells were significant ($P < 0.001$, one-way ANOVA).

Figs. 6(A-B) show that anti-MBP T cells increase the compound action potential (CAP) amplitudes of injured optic nerves. Immediately after optic nerve injury, rats were injected with either PBS or activated anti-MBP T cells (T_{MBP}). Two weeks later, the CAPs of injured (Fig. 6A) and uninjured (Fig. 6B) nerves were recorded. There were no significant differences in mean CAP amplitudes between uninjured nerves obtained from PBS-injected and T cell-injected rats ($n=8$; $p=0.8$, Student's t-test). The neuroprotective effect of anti-MBP T cells (relative to PBS) on the injured nerve on day 14 after injury was significant ($n=8$; $p=0.009$, Student's t-test).

Fig. 7 illustrates inhibition of secondary degeneration after optic nerve crush injury in adult rats. See text,

Section 8, for experimental details. Rats were injected intradermally through the footpads with a 21-mer peptide based on amino acid residues 35-55 (MOG p35-55) of myelin/oligodendrocyte glycoprotein (chemically synthesized at the Weizmann Institute, Israel) (50 μ g/animal) or PBS ten days prior to optic nerve crush injury or MOG p35-55 in the absence of crush injury. MOG p35-55 was administered with Incomplete Freund's Adjuvant. Surviving optic nerve fibers were monitored by retrograde labeling of retinal ganglion cells (RGCs). The number of RGCs in rats injected with PBS or MOG p35-55 was expressed as a percentage of the total number of neurons in rats injected with MOG p35-55 in the absence of crush injury.

Fig. 8 illustrates inhibition in adult rats of secondary degeneration after optic nerve crush injury by MBP. See text, Section 9, for experimental details. MBP (Sigma, Israel) (1 mg in 0.5 ml saline) was administered orally to adult rats by gavage using a blunt needle. MBP was administered 5 times, i.e., every third day beginning 2 weeks prior to optic nerve crush injury. Surviving optic nerve fibers were monitored by retrograde labeling of retinal ganglion cells (RGCs). The number of RGCs in treated rats was expressed as a percentage of the total number of neurons in untreated rats following the injury.

Fig. 9 shows the nucleotide sequence of rat myelin basic protein gene, SEQ ID NO: _____, Genbank accession number M25889 (Schaich et al., 1986, *Biol. Chem.* 367, 825-834).

Fig. 10 shows the nucleotide sequence of human myelin basic protein gene, SEQ ID NO: _____, Genbank accession number M13577 (Kamholz et al., 1986, *Proc. Natl. Acad. Sci. U.S.A.* 83 (13), 4962-4966).

Figs. 11(A-F) show the nucleotide sequences of human myelin proteolipid protein gene exons 1-7, SEQ ID NO: _____, Genbank accession numbers M15026-M15032 respectively (Diehl et

al., [published erratum appears in *Proc Natl Acad Sci U S A*, 1991, 86(6):617-8] *Proc. Natl. Acad. Sci. U.S.A.* 83 (24), 9807-9811 (1986)).

5 **Fig. 12** shows the nucleotide sequence of human myelin oligodendrocyte glycoprotein gene, SEQ ID NO: _____, Genbank accession number Z48051 (Roth et al., submitted (17-JAN-1995) Roth, CNRS UPR 8291, CIGH, CHU Purpan, Toulouse, France, 31300; Gonzalez et al., 1996, *Mol. Phylogenet. Evol.* 6, 63-71).

10 **Fig. 13** shows the nucleotide sequence of rat proteolipid protein and variant, SEQ ID NO: _____, Genbank accession number M16471 (Nave et al., 1987, *Proc. Natl. Acad. Sci. U.S.A.* 84, 5665-5669).

15 **Fig. 14** shows the nucleotide sequence of rat myelin-associated glycoprotein, SEQ ID NO: _____, Genbank accession number M14871 (Arquint et al., 1987, *Proc. Natl. Acad. Sci. U.S.A.* 84, 600-604).

Fig. 15 shows the amino acid sequence of human myelin basic protein, SEQ ID NO: _____, Genbank accession number 307160 (Kamholz et al., 1986, *Proc. Natl. Acad. Sci. U.S.A.* 83 (13), 4962-4966).

20 **Fig. 16** shows the amino acid sequence of human proteolipid protein, SEQ ID NO: _____, Genbank accession number 387028.

Fig. 17 shows the amino acid sequence of human myelin oligodendrocyte glycoprotein, SEQ ID NO: _____, Genbank accession number 793839 (Roth et al., 1995, *Genomics* 28 (2), 241-250; Roth Submitted (17-JAN-1995) Roth CNRS UPR 8291, CIGH, CHU Purpan, Toulouse, France, 31300; Gonzalez et al., 1996, *Mol. Phylogenet. Evol.* 6, 63-71).

5. DETAILED DESCRIPTION OF THE INVENTION

30 Merely for ease of explanation, the detailed description of the present invention is divided into the following subsections: (1) non-recombinant, NS-specific antiself activated

T-cells; (2) NS-specific antigens, peptides derived therefrom and derivatives thereof; (3) nucleotide sequences encoding NS-specific antigens and peptides derived therefrom; (4) therapeutic uses of non-recombinant, NS-specific antise-
5 activated T-cells, NS-specific antigens, peptides derived therefrom and derivatives thereof, and nucleotide sequences encoding NS-specific antigens and peptides derived therefrom;
and (5) formulations and modes of administration of non-recombinant, NS-specific antise-
10 specific antigens, peptides derived therefrom and derivatives thereof, and nucleotide sequences encoding NS-specific antigens and peptides derived therefrom.

5.1 NS-SPECIFIC ANTISELF ACTIVATED T-CELLS

15 NS-specific antise-activated T-cells (ATCs) can be used for ameliorating or inhibiting the effects of injury or disease of the CNS or PNS that result in NS degeneration or for promoting regeneration in the NS, in particular the CNS.

The NS-specific activated T-cells are preferably
20 autologous, most preferably of the CD4 and/or CD8 phenotypes, but they may be also allogeneic T-cells from related donors, e.g. siblings, parents, children, or HLA-matched or partially matched, semi-allogeneic or fully allogeneic donors.

The NS-specific antise-activated T-cells are preferably
25 non-attenuated, although attenuated NS-specific activated T-cells may be used. T-cells may be attenuated using methods well known in the art, including but not limited to, by gamma-irradiation, e.g. 1.5-10.0 Rads (Ben-Nun, A., Wekerle, H. and Cohen, I.R., *Nature* 292:60-61 (1981); Ben-Nun, A. and Cohen, I.R., *J. Immunol.* 129:303-308 (1982)); and/or by pressure
30 treatment, for example as described in U.S. Patent No. 4,996,194 (Cohen et al.); and/or by chemical cross-linking with

an agent such as formaldehyde, glutaraldehyde and the like, for example as described in U.S. Patent No. 4,996,194 (Cohen et al.); and/or by cross-linking and photoactivation with light with a photoactivatable psoralen compound, for example as
5 described in U.S. Patent No. 5,114,721 (Cohen et al.); and/or by a cytoskeletal disrupting agent such as cytochalsin and colchicine, for example as described in U.S. Patent No. 4,996,194 (Cohen et al.). In a preferred embodiment the NS-specific antiseif activated T-cells are isolated as described
10 below. T-cells can be isolated and purified according to methods known in the art (Mor and Cohen, 1995, *J. Immunol.* 155:3693-3699). For an illustrative example, see Section 6.1.

Circulating T-cells of a subject which recognize myelin basic protein or another NS antigen such as the amyloid
15 precursor protein are isolated and expanded using known procedures. In order to obtain NS-specific antiseif activated T-cells, T-cells are isolated and the NS-specific ATCs are then expanded by a known procedure (Burns et al., *Cell Immunol.* 81:435 (1983); Pette et al., *Proc. Natl. Acad. Sci. USA* 87:7968
20 (1990); Mortin et al., *J. Immunol.* 145:540 (1990); Schluesener et al., *J. Immunol.* 135:3128 (1985); Suruhan-Dires Keneli et al., *Euro. J. Immunol.* 23:530 (1993) which are incorporated herein by reference in their entirety.

The isolated T-cells may be activated by exposure of the
25 cells to one or more of a variety of natural or synthetic NS-specific antigens or epitopes, including but not limited to, myelin basic protein (MBP), myelin oligodendrocyte glycoprotein (MOG), proteolipid protein (PLP), myelin-associated glycoprotein (MAG), S-100, β -amyloid, Thy-1, P0, P2 and
30 neurotransmitter receptors. In a preferred embodiment, the isolated T cells are activated by one or more cryptic epitopes, including but limited to the following MBP peptides: p11-30,

p51-70, p91-110, p131-150, and p151-170.

During *ex vivo* activation of the T-cells, the T-cells may be activated by culturing them in medium to which at least one suitable growth promoting factor has been added. Growth
5 promoting factors suitable for this purpose include, without limitation, cytokines, for instance tumor necrosis factor α (TNF- α), interleukin 2 (IL-2), and interleukin 4 (IL-4).

In an embodiment, the activated T-cells endogenously produce a substance that ameliorates the effects of injury or
10 disease in the CNS.

In another embodiment, the activated T-cells endogenously produce a substance that stimulates other cells, including, but not limited to, transforming growth factor- β (TGF- β), nerve growth factor (NGF), neurotrophic factor 3 (NT-3), neurotrophic
15 factor 4/5 (NT-4/5), brain-derived neurotrophic factor (BDNF); interferon- γ (IFN- γ), interleukin-6 (IL-6), wherein the other cells, directly or indirectly, ameliorate the effects of injury or disease.

Following their proliferation *in vitro*, the T-cells are
20 administered to a mammalian subject. In a preferred embodiment, the T-cells are administered to a human subject. T-cell expansion is preferably performed using peptides corresponding to sequences in a non-pathogenic, NS-specific, self protein.

25 A subject can initially be immunized with a NS-specific antigen using a non-pathogenic peptide of the self protein. A T-cell preparation can be prepared from the blood of such immunized subjects, preferably from T-cells selected for their specificity towards the NS-specific antigen. The selected T-
30 cells can then be stimulated to produce a T-cell line specific to the self-antigen (Ben-Nun et al., *J. Immunol.* 129:303 (1982)).

The NS-specific antigen may be a purified antigen or a crude NS preparation, as will be described below.

NS-specific antigen activated T-cells, obtained as described above, can be used immediately or may be preserved 5 for later use, e.g. by cryopreservation as described below. NS-specific antiseif activated T-cells may also be obtained using previously cryopreserved T-cells, *i.e.*, after thawing the cells, the T-cells may be incubated with NS-specific antigen, optimally together with thymocytes, to obtain a preparation of 10 NS-specific ATCs.

As will be evident to those skilled in the art, the T-cells can be preserved, e.g. by cryopreservation, either before or after culture.

Cryopreservation agents which can be used include but are 15 not limited to dimethyl sulfoxide (DMSO) (Lovelock and Bishop, 1959, Nature 183:1394-1395; Ashwood-Smith, 1961, Nature 190:1204-1205), glycerol, polyvinylpyrrolidone (Rinfret, 1960, Ann. N.Y. Acad. Sci. 85:576), polyethylene glycol (Sloviter and Ravdin, 1962, Nature 196:548), albumin, dextran, sucrose, 20 ethylene glycol, i-erythritol, D-ribitol, D-mannitol (Rowe et al., 1962, Fed. Proc. 21:157), D-sorbitol, i-inositol, D-lactose, choline chloride (Bender et al., 1960, J. Appl. Physiol. 15:520), amino acids (Phan The Tran and Bender, 1960, Exp. Cell Res. 20:651), methanol, acetamide, glycerol 25 monoacetate (Lovelock, 1954, Biochem. J. 56:265), inorganic salts (Phan The Tran and Bender, 1960, Proc. Soc. Exp. Biol. Med. 104:388; Phan The Tran and Bender, 1961, in Radiobiology, Proceedings of the Third Australian Conference on Radiobiology, Ilbery, P.L.T., ed., Butterworth, London, p. 59), and DMSO 30 combined with hydroxyethyl starch and human serum albumin (Zaroulis and Leiderman, 1980, Cryobiology 17:311-317).

A controlled cooling rate is critical. Different

cryoprotective agents (Rapatz et al., 1968, Cryobiology 5(1):18-25) and different cell types have different optimal cooling rates. See, e.g., Rowe and Rinfret, 1962, Blood 20:636; Rowe, 1966, Cryobiology 3(1):12-18; Lewis et al., 1967, 5 Transfusion 7(1):17-32; and Mazur, 1970, Science 168:939-949 for effects of cooling velocity on survival of cells and on their transplantation potential. The heat of fusion phase where water turns to ice should be minimal. The cooling procedure can be carried out by use of, e.g., a programmable 10 freezing device or a methanol bath procedure.

Programmable freezing apparatuses allow determination of optimal cooling rates and facilitate standard reproducible cooling. Programmable controlled-rate freezers such as Cryomed or Planar permit tuning of the freezing regimen to the desired 15 cooling rate curve.

After thorough freezing, cells can be rapidly transferred to a long-term cryogenic storage vessel. In one embodiment, samples can be cryogenically stored in mechanical freezers, such as freezers that maintain a temperature of about -80°C or 20 about -20°C. In a preferred embodiment, samples can be cryogenically stored in liquid nitrogen (-196°C) or its vapor. Such storage is greatly facilitated by the availability of highly efficient liquid nitrogen refrigerators, which resemble large Thermos containers with an extremely low vacuum and 25 internal super insulation, such that heat leakage and nitrogen losses are kept to an absolute minimum.

Considerations and procedures for the manipulation, cryopreservation, and long term storage of T-cells can be found, for example, in the following references, incorporated 30 by reference herein: Gorin, 1986, Clinics in Haematology 15(1):19-48; Bone-Marrow Conservation, Culture and Transplantation, Proceedings of a Panel, Moscow, July 22-26,

1968, International Atomic Energy Agency, Vienna, pp. 107-186.

Other methods of cryopreservation of viable cells, or modifications thereof, are available and envisioned for use, e.g., cold metal-mirror techniques. See Livesey and Linner, 5 1987, Nature 327:255; Linner et al., 1986, J. Histochem. Cytochem. 34(9):1123-1135; see also U.S. Patent No. 4,199,022 by Senken et al., U.S. Patent No. 3,753,357 by Schwartz, U.S. Patent No. 4,559,298 by Fahy.

Frozen cells are preferably thawed quickly (e.g., in a 10 water bath maintained at 37-41°C) and chilled immediately upon thawing. It may be desirable to treat the cells in order to prevent cellular clumping upon thawing. To prevent clumping, various procedures can be used, including but not limited to the addition before or after freezing of DNase (Spitzer et al., 15 1980, Cancer 45:3075-3085), low molecular weight dextran and citrate, hydroxyethyl starch (Stiff et al., 1983, Cryobiology 20:17-24), or acid citrate dextrose (Zaroulis and Leiderman, 1980, Cryobiology 17:311-317), etc.

The cryoprotective agent, if toxic in humans, should be 20 removed prior to therapeutic use of the thawed T-cells. One way in which to remove the cryoprotective agent is by dilution to an insignificant concentration.

Once frozen T-cells have been thawed and recovered, they are used to promote axonal regeneration as described herein 25 with respect to non-frozen T-cells.

5.2 NS-SPECIFIC ANTIGENS AND PEPTIDES DERIVED THEREFROM

Pharmaceutical compositions comprising a NS-specific 30 antigen or peptide derived therefrom or derivative thereof can be used for preventing or inhibiting the effects of injury or disease that result in NS degeneration or for promoting nerve regeneration in the NS, particularly in the CNS. Additionally,

NS-specific antigens or peptides derived therefrom or derivatives thereof may be used for *in vivo* or *in vitro* activation of antiself T-cells. In an embodiment, the NS-specific antigen is an isolated or purified antigen. In an
5 embodiment, methods of promoting nerve regeneration or of preventing or inhibiting the effects of CNS or PNS injury or disease comprise administering NS-specific antigen or a peptide derived therefrom or derivative thereof to a mammal wherein the NS-specific antigen or peptide derived therefrom or derivative
10 thereof activates T-cells *in vivo* to produce a population of T-cells that accumulate at a site of injury or disease of the CNS or PNS.

The NS-specific antigen may be an antigen obtained from NS tissue, preferably from tissue at a site of CNS injury or
15 disease. The NS-specific antigen may be isolated and purified by standard methods including chromatography (e.g., ion exchange, affinity, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of antigens. The
20 functional properties may be evaluated using any suitable assay. In the practice of the invention, natural or synthetic NS-specific antigens or epitopes include, but are not limited to, MBP, MOG, PLP, MAG, S-100, β -amyloid, Thy-1, P0, P2 and a neurotransmitter receptor.

25 Specific illustrative examples of useful NS-specific antigens include but are not limited to, human MBP, depicted in Fig. 15 (SEQ ID NO: _____); human proteolipid protein, depicted in Fig. 16 (SEQ ID NO: _____); and human oligodendrocyte glycoprotein, depicted in Fig. 17 (SEQ ID NO: _____).

30 In a preferred embodiment, peptides derived from NS-specific, self antigens or derivatives of NS-specific antigens activate T-cells, but do not induce an autoimmune disease. An

example of such peptide is a peptide comprising amino acids 51-70 of myelin basic protein. SEQ ID NO:___ (Kamholz et al., 1986, Proc. Natl. Acad. Sci. USA 83:4962-4966, GenBank accession number M13577; Roth et al., 1987, J. Neurosci. Res. 5 17(4):321-328, GenBank accession number M30516).

In addition, a NS-specific antigen may be a crude NS-tissue preparation, e.g., derived from NS tissue obtained from mammalian NS. Such a preparation may include cells, both living or dead cells, membrane fractions of such cells or 10 tissue, etc.

A NS-specific antigen may be obtained by a NS biopsy or necropsy from a mammal including, but not limited to, from a site of CNS injury; from cadavers; from cell lines grown in culture. Additionally, a NS-specific antigen may be a protein 15 obtained by genetic engineering, chemically synthesized, etc.

In addition to NS-specific antigens, the invention also relates to peptides derived from NS-specific antigens or derivatives including chemical derivatives and analogs of NS-specific antigens which are functionally active, i.e., they are 20 capable of displaying one or more known functional activities associated with a full-length NS-specific antigen. Such functional activities include but are not limited to antigenicity [ability to bind (or compete with a CNS-antigen for binding) to an anti-NS-specific antibody], immunogenicity 25 (ability to generate antibody which binds to a NS-specific protein), and ability to interact with T-cells, resulting in activation comparable to that obtained using the corresponding full-length antigen.

A peptide derived from a CNS-specific or PNS-specific 30 antigen has a sequence comprised within the antigen sequence and is either: (1) an immunogenic peptide, i.e., a peptide that can elicit a human T-cell response detected by T-cell

proliferation or by cytokine (e.g. interferon (IFN)- γ , interleukin (IL)-2, IL-4 or IL-10) production or (2) a "cryptic epitope" (also designated herein as "immunosilent" or "nonimmunodominant" epitope), i.e., a peptide that by itself
5 can induce a T-cell immune response that is not induced by the whole antigen protein (see Moalem et al., 1999, Nature Med. 5(1)). Cryptic epitopes for use in the present invention include, but are not limited to, peptides of the myelin basic protein sequence: peptide p11-30, p51-70, p91-110, p131-150,
10 and p151-170. Other peptides can be identified by their capacity to elicit a human T-cell response detected by T-cell proliferation or by cytokine (e.g. IFN- γ , IL-2, IL-4, or IL-10) production.

In a specific embodiment of the invention, peptides
15 consisting of or comprising a fragment of a NS-specific antigen consisting of at least 10 (contiguous) amino acids of the NS-specific antigen is provided. In other embodiments, the fragment consists of at least 20 contiguous amino acids or 50 contiguous amino acids of the NS-specific antigen.

20 Derivatives of a NS-specific antigen also include but are not limited to those molecules comprising regions that are substantially homologous to the full-length antigen or fragments thereof (e.g., in various embodiments, at least 60% or 70% or 80% or 90% or 95% identity over an amino acid
25 sequence of identical size or when compared to an aligned sequence in which the alignment is done by a computer homology program known in the art) or whose encoding nucleic acid is capable of hybridizing to a coding nucleotide sequence of the full-length NS-specific antigen, under high stringency,
30 moderate stringency, or low stringency conditions.

Computer programs for determining homology may include but are not limited to TBLASTN, BLASTP, FASTA, TFASTA, and CLUSTALW

(Pearson and Lipman, 1988, Proc. Natl. Acad. Sci. USA 85(8):2444-8; Altschul et al., 1990, J. Mol. Biol. 215(3):403-10; Thompson, et al., 1994, Nucleic Acids Res. 22(22):4673-80; Higgins, et al., 1996, Methods Enzymol 266:383-402; Altschul, 5 et al., 1990, J. Mol. Biol. 215(3):403-10).

The NS-specific antigen derivatives of the invention can be produced by various methods known in the art. The manipulations which result in their production can occur at the gene or protein level. For example, a cloned gene sequence can 10 be modified by any of numerous strategies known in the art (Maniatis, T., 1990, Molecular Cloning, A Laboratory Manual, 2d ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York). The sequence can be cleaved at appropriate sites with restriction endonuclease(s), followed by further enzymatic 15 modification if desired, isolated, and ligated *in vitro*.

Additionally, the coding nucleic acid sequence can be mutated *in vitro* or *in vivo*, to create and/or destroy translation, initiation, and/or termination sequences, or to create variations in coding regions and/or form new restriction 20 endonuclease sites or destroy preexisting ones, to facilitate further *in vitro* modification. Any technique for mutagenesis known in the art can be used, including but not limited to, chemical mutagenesis, *in vitro* site-directed mutagenesis (Hutchinson, C., et al., 1978, J. Biol. Chem 253:6551), etc.

25 Manipulations may also be made at the protein level. Included within the scope of the invention are derivatives which are differentially modified during or after translation, e.g., by glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, 30 proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc. Any of numerous chemical modifications may be carried out by known techniques, including but not

limited to specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease, NaBH_4 ; acetylation, formylation, oxidation, reduction; metabolic synthesis in the presence of tunicamycin; etc.

5 In addition, derivatives of a NS-specific antigen can be chemically synthesized. For example, a peptide corresponding to a portion of an antigen which comprises the desired domain or which mediates the desired activity can be synthesized by use of a peptide synthesizer. Furthermore, if desired,
10 nonclassical amino acids or chemical amino acid analogs can be introduced as a substitution or addition into the amino acid sequence. Non-classical amino acids include but are not limited to the D-isomers of the common amino acids, α -amino isobutyric acid, 4-aminobutyric acid, Abu, 2-amino butyric
15 acid, γ -Abu, ϵ -Ahx, 6-amino hexanoic acid, Aib, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, β -alanine, fluoro-amino acids, designer
20 amino acids such as β -methyl amino acids, C α -methyl amino acids, N α -methyl amino acids, and amino acid analogs in general. Furthermore, the amino acid can be D (dextrorotary) or L (levorotary).

The functional activity of NS-specific antigens and
25 peptides derived therefrom and derivatives thereof can be assayed by various methods known in the art, including, but not limited to T-cell proliferation assays (Mor and Cohen, 1995, J. Immunol. 155:3693-3699).

A NS-specific antigen or peptide derived therefrom or
30 derivative thereof may be kept in solution or may be provided in a dry form, e.g. as a powder or lyophilizate, to be mixed with appropriate solution prior to use.

5.3 NUCLEOTIDE SEQUENCES ENCODING NS-ANTIGENS AND PEPTIDES DERIVED THEREFROM

Compositions comprising a nucleotide sequence encoding a NS-specific antigen or peptide derived therefrom can be used for preventing or inhibiting the effects of injury or disease that result in CNS or PNS degeneration or for promoting nerve regeneration in the CNS or PNS. Specific illustrative examples of useful nucleotide sequences encoding NS-specific antigens or peptides derived from a NS-specific antigen, include but are not limited to nucleotide sequences encoding rat myelin basic protein (MBP) peptides, depicted in Fig. 9 (SEQ ID NO: ____); human MBP, depicted in Fig. 10 (SEQ ID NO: ____); human myelin PLP, depicted in Figs. 11(A-F) (SEQ ID NO: ____); human MOG, depicted in Fig. 12 (SEQ ID NO: ____); rat PLP and variant, depicted in Fig. 13 (SEQ ID NO: ____); and rat MAG, depicted in Fig. 14 (SEQ ID NO: ____).

5.4 THERAPEUTIC USES

The compositions described in Sections 5.1 through 5.3 may be used to promote nerve regeneration or to prevent or inhibit secondary degeneration which may otherwise follow primary NS injury, e.g. blunt trauma, penetrating trauma, hemorrhagic stroke, ischemic stroke or damages caused by surgery such as tumor excision. In addition, such compositions may be used to ameliorate the effects of disease that result in a degenerative process, e.g. degeneration occurring in either grey or white matter (or both) as a result of various diseases or disorders which are not recognized by those of reasonable skill in the art as being autoimmune diseases or disorders including, without limitation: Diabetic neuropathy, senile dementias, Alzheimer's disease, Parkinson's Disease, facial nerve (Bell's) palsy, glaucoma, Huntington's chorea, amyotrophic lateral sclerosis (ALS), non-arteritic optic neuropathy, intervertebral

disc herniation, vitamin deficiency, prion diseases such as Creutzfeldt-Jakob disease, carpal tunnel syndrome, peripheral neuropathies associated with various diseases, including but not limited to, uremia, porphyria, hypoglycemia, Sjörgren-
5 Larsson syndrome, acute sensory neuropathy, chronic ataxic neuropathy, biliary cirrhosis, primary amyloidosis, obstructive lung diseases, acromegaly, malabsorption syndromes, polycythemia vera, IgA and IgG gammopathies, complications of various drugs (e.g. metronidazole) and toxins (e.g. alcohol or
10 organophosphates), Charcot-Marie-Tooth disease, ataxia telangiectasia, Friedreich's ataxia, amyloid polyneuropathies, adrenomyeloneuropathy, Giant axonal neuropathy, Refsum's disease, Fabry's disease, lipoproteinemia, etc.

In a preferred embodiment, the NS-specific antiself
15 activated T-cells, the NS-specific antigens, peptides derived therefrom, derivatives thereof or the nucleotides encoding said antigens, or peptides or any combination thereof of the present invention are used to treat diseases or disorders which are not autoimmune diseases or neoplasias. In a preferred embodiment,
20 the compositions of the present invention are administered to a human subject.

5.5 FORMULATIONS AND ADMINISTRATION

Pharmaceutical compositions for use in accordance with the
25 present invention may be formulated in conventional manner using one or more physiologically acceptable carriers or excipients. The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the composition and not deleterious to the recipient thereof.

30 The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. The carriers in the pharmaceutical composition

may comprise a binder, such as microcrystalline cellulose, polyvinylpyrrolidone (polyvidone or povidone), gum tragacanth, gelatine, starch, lactose or lactose monohydrate; a disintegrating agent, such as alginic acid, maize starch and
5 the like; a lubricant or surfactant, such as magnesium stearate, or sodium lauryl sulphate; a glidant, such as colloidal silicon dioxide; a sweetening agent, such as sucrose or saccharin; and/or a flavoring agent, such as peppermint, methyl salicylate, or orange flavoring.

10 Methods of administration include, but are not limited to, parenteral, e.g. intravenous, intraperitoneal, intramuscular, subcutaneous, mucosal (e.g., oral, intranasal, buccal, vaginal, rectal, intraocular), intrathecal and intradermal routes. Administration can be systemic or local.

15 For oral administration, the pharmaceutical preparation may be in liquid form, for example, solutions, syrups or suspensions, or may be presented as a drug product for reconstitution with water or other suitable vehicle before use. Such liquid preparations may be prepared by conventional means
20 with pharmaceutically acceptable additives such as suspending agents (e.g., sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (e.g., lecithin or acacia); non-aqueous vehicles (e.g., almond oil, oily esters, or fractionated vegetable oils); and preservatives
25 (e.g., methyl or propyl-p-hydroxybenzoates or sorbic acid). The pharmaceutical compositions may take the form of, for example, tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (e.g., pregelatinized maize starch, polyvinyl
30 pyrrolidone or hydroxypropyl methylcellulose); fillers (e.g., lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (e.g., magnesium stearate, talc or

silica); disintegrants (e.g., potato starch or sodium starch glycolate); or wetting agents (e.g., sodium lauryl sulphate). The tablets may be coated by methods well-known in the art.

Preparations for oral administration may be suitably
5 formulated to give controlled release of the active compound.

For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

The compositions may be formulated for parenteral administration by injection, e.g., by bolus injection or
10 continuous infusion. Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents
15 such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use.

The compositions may also be formulated in rectal
20 compositions such as suppositories or retention enemas, e.g., containing conventional suppository bases such as cocoa butter or other glycerides.

For administration by inhalation, the compositions for use according to the present invention are conveniently delivered
25 in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may
30 be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, e.g., gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the

compound and a suitable powder base such as lactose or starch.

In a preferred embodiment, compositions comprising NS-specific antiseif activated T-cells, a NS-specific antigen or peptide derived therefrom, or derivative thereof, or a
5 nucleotide sequence encoding such antigen or peptide are formulated in accordance with routine procedures as pharmaceutical compositions adapted for intravenous or intraperitoneal administration to human beings. Typically, compositions for intravenous administration are solutions in
10 sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together. Where the composition is to be
15 administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water or saline for injection can be provided so that the ingredients may be mixed prior to administration.

20 Pharmaceutical compositions comprising NS-specific antigen or peptide derived therefrom or derivative thereof may optionally be administered with an adjuvant, such as Incomplete Freund's Adjuvant.

The invention also provides a pharmaceutical pack or kit
25 comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention.

In a preferred embodiment, the pharmaceutical compositions of the invention are administered to a mammal, preferably a
30 human, shortly after injury or detection of a degenerative lesion in the NS. The therapeutic methods of the invention may comprise administration of a NS-specific antiseif activated T-

cell, or a NS-specific antigen or peptide derived therefrom or derivative thereof, or a nucleotide sequence encoding such antigen or peptide or any combination thereof. The NS-specific antigen may be administered before, concurrently or after
5 administration of NS-specific antiseif activated T-cells, a peptide derived from a NS-specific antigen or derivative thereof or a nucleotide sequence encoding such antigen or peptide.

In an embodiment, the compositions of the invention are
10 administered in combination with one or more of the following:
(a) mononuclear phagocytes, preferably cultured monocytes (as described in PCT publication No. WO 97/09985, which is incorporated herein by reference in its entirety), that have been stimulated to enhance their capacity to promote axonal
15 regeneration; (b) a neurotrophic factor such as acidic fibroblast growth factor; and (c) an anti-inflammatory therapeutic substance (*i.e.*, an anti-inflammatory steroid, such as dexamethasone or methylprednisolone, or a non-steroidal anti-inflammatory agent or drug, such as aspirin, indomethacin,
20 ibuprofen, fenoprofen, ketoprofen or haproxen, or an anti-inflammatory peptide, such as Thr-Lys-Pro (TKP)).

In an embodiment, mononuclear phagocyte cells according PCT Publication No. WO 97/09985 and U.S. patent application Serial No. 09/041,280, filed March 11, 1998, are injected into
25 the site of injury or lesion within the CNS, either concurrently, prior to, or following parenteral administration of NS-specific antiseif activated T-cells, a NS-specific antigen or peptide derived therefrom or derivative thereof, or a nucleotide sequence encoding such antigen or peptide.

In an embodiment, administration of NS-specific activated
30 T-cells, a NS-specific antigen or peptide derived therefrom or derivative thereof, or a nucleotide sequence encoding such antigen or peptide, may be administered as a single dose or may

be repeated, preferably at 2 week intervals and then successively longer intervals once a month, once a quarter, once every six months, etc. The course of treatment may last several months, several years or occasionally also through the life-time of the individual, depending on the condition or disease which is being treated. In the case of a CNS injury, the treatment may range between several days to months or even years, until the condition has stabilized and there is no or only a limited risk of development of secondary degeneration. In chronic human diseases or conditions such as Alzheimer's disease or Parkinson's disease, the therapeutic treatment in accordance with the invention may be for life.

As will be evident to those of skill in the art, the therapeutic effect depends at times on the condition or disease to be treated, on the individual's age and health condition, on other physical parameters (e.g. gender, weight, etc.) of the individual, as well as on various other factors, e.g. whether the individual is taking other drugs, etc.

The optimal dose of the therapeutic compositions comprising NS-specific antiseif activated T-cells of the invention is proportional to the number of nerve fibers affected by CNS injury or disease at the site being treated. In a preferred embodiment, the dose ranges from about 5×10^6 to about 10^7 for treating a lesion affecting about 10^5 nerve fibers, such as a complete transection of a rat optic nerve, and ranges from about 10^7 to about 10^8 for treating a lesion affecting about 10^6 - 10^7 nerve fibers, such as a complete transection of a human optic nerve. As will be evident to those of skill in the art, the dose of T-cells can be scaled up or down in proportion to the number of nerve fibers thought to be affected at the lesion or site of injury being treated.

The following examples illustrate certain features of the present invention but are not intended to limit the scope of the present invention.

6. **EXAMPLE: ACCUMULATION OF ACTIVATED
T-CELLS IN INJURED OPTIC NERVE**

5 6.1 **MATERIALS AND METHODS**

6.1.1 **ANIMALS**

Female Lewis rats were supplied by the Animal Breeding Center of the Weizmann Institute of Science (Rehovot, IL), matched for age (8-12 weeks) and housed four to a cage in a
10 light and temperature-controlled room.

6.1.2 **MEDIA**

The T-cell proliferation medium contained the following:
Dulbecco's modified Eagle's medium (DMEM, Biological
15 Industries, Israel) supplemented with 2mM L-glutamine (L-Glu, Sigma, USA), 5×10^{-5} M 2-mercaptoethanol (2-ME, Sigma), penicillin (100 IU/ml; Biological Industries), streptomycin (100 μ g/ml; Biological Industries), sodium pyruvate (1 mM; Biological Industries), non-essential amino acids (1 ml/100 ml; Biological Industries) and autologous rat serum 1% (vol/vol) (Mor et al.,
20 Clin. Invest., 85:1594 (1990)). Propagation medium contained: DMEM, 2-ME, L-Glu, sodium pyruvate, non-essential amino acids and antibiotics in the same concentration as above with the addition of 10% fetal calf serum (FCS), and 10% T-cell growth factor (TCGF) obtained from the supernatant of concanavalin A-
25 stimulated spleen cells (Mor et al., *supra*, 1990).

6.1.3 **ANTIGENS**

Myelin basic protein (MBP) from the spinal cords of guinea pigs was prepared as described (Hirshfeld, et al., 1970, FEBS
30 Lett. 7:317). Ovalbumin was purchased from Sigma (St. Louis, Missouri). The p51-70 of the rat 18.5kDa isoform of MBP (sequence: APKRGSGKDSHTRTTHYG) SEQ ID NO:____ and the p277

peptide of the human hsp60 (sequence: VLGGGCALLRCPALDSLTPANED)
SEQ ID NO:_____ (Elias, et al., 1991, Proc. Natl. Acad. Sci. USA
88, 3088-91) were synthesized using the 9-
fluorenylmethoxycarbonyl technique with an automatic multiple
5 peptide synthesizer (AMS 422, ABIMED, Langenfeld, Germany).
The purity of the peptides was analyzed by HPLC and amino acid
composition.

6.1.4 T-CELL LINES

10 T-cell lines were generated from draining lymph node cells
obtained from Lewis rats immunized with an antigen (described
above in Section 6.1.3). The antigen was dissolved in PBS
(1mg/ml) and emulsified with an equal volume of incomplete
Freund's adjuvant (Difco Laboratories, Detroit, Michigan)
supplemented with 4 mg/ml *Mycobacterium tuberculosis* (Difco
15 Laboratories, Detroit, Michigan). The emulsion (0.1 ml) was
injected into hind foot pads of the rats. Ten days after the
antigen was injected, the rats were killed and draining lymph
nodes were surgically removed and dissociated. The cells were
washed and activated with the antigen (10µg/ml) in
20 proliferation medium (described above in Section 6.1.2). After
incubation for 72 h at 37°C, 90% relative humidity and 7% CO₂,
the cells were transferred to propagation medium (described
above in Section 6.1.2). Cells were grown in propagation
medium for 4-10 days before being re-exposed to antigen
25 (10µg/ml) in the presence of irradiated (2000 rad) thymus cells
(10⁷ cells/ml) in proliferation medium. The T-cell lines were
expanded by repeated re-exposure and propagation.

6.1.5 CRUSH INJURY OF RAT OPTIC NERVE

30 Crush injury of the optic nerve was performed as
previously described (Duvdevani et al., 1990, Neurol. Neurosci.
2:31-38). Briefly, rats were deeply anesthetized by i.p.

injection of Rompun (xylazine, 10 mg/kg; Vitamed, Israel) and Vetalar (ketamine, 50 mg/kg; Fort Dodge Laboratories, Fort Dodge, Iowa). Using a binocular operating microscope, a lateral canthotomy was performed in the right eye and the conjunctiva was incised lateral to the cornea. After separation of the retractor bulbi muscles, the optic nerve was exposed intraorbitally by blunt dissection. Using calibrated cross-action forceps, a moderate crush injury was inflicted on the optic nerve, 2 mm from the eye (Duvdevani et al., *Instructure Neurology and Neuroscience*, 2:31, 1990). The contralateral nerve was left undisturbed and was used as a control.

6.1.6 IMMUNOCYTOCHEMISTRY OF T-CELLS

Longitudinal cryostat nerve sections (20 μ m thick) were picked up onto gelatin glass slides and frozen until preparation for fluorescent staining. Sections were thawed and fixed in ethanol for 10 minutes at room temperature, washed twice with double-distilled water (ddH₂O), and incubated for 3 minutes in PBS containing 0.05% polyoxyethylene-sorbitan monolaurate (Tween-20; Sigma, USA). Sections were then incubated for 1 hr at room temperature with a mouse monoclonal antibody directed against rat T-cell receptor (TCR) (1:100, Hunig et al., *J. Exp. Med.*, 169:73, 1989), in PBS containing 3% FCS and 2% BSA. After three washes with PBS containing 0.05% Tween-20, the sections were incubated with fluorescein isothiocyanate-conjugated goat anti-mouse IgG (with minimal cross-reaction to rat, human, bovine and horse serum proteins) (Jackson ImmunoResearch, West Grove, Pennsylvania) for 1 hr at room temperature. The sections were then washed with PBS containing Tween-20 and treated with glycerol containing 1,4-diazobicyclo-(2,2,2) octane (Sigma), to inhibit quenching of fluorescence. The sections were viewed with a Zeiss microscope

and cells were counted. Staining in the absence of first antibody was negative.

5 6.2 RESULTS

Fig. 1 shows accumulation of T-cells measured immuno-histochemically. The number of T cells was considerably higher in injured nerves of rats injected with anti-MBP, anti-OVA or anti-p277 cells; statistical analysis (one-way ANOVA) showed significant differences between T cell numbers in injured optic
10 nerves of rats injected with anti-MBP, anti-OVA, or anti-p277 T cells and in injured optic nerves of rats injected with PBS ($P < 0.001$); and between injured optic nerves and uninjured optic nerves of rats injected with anti-MBP, anti-OVA, or anti-p277 T cells ($P < 0.001$).

15

7. EXAMPLE: NEUROPROTECTION BY AUTOIMMUNE ANTI-MBP T-CELLS

7.1 MATERIALS AND METHODS

Animals, media, antigens, crush injury of rat optic nerve, sectioning of nerves, T-cell lines, and immunolabeling of nerve
20 sections are described in Section 6, *supra*.

7.1.1 RETROGRADE LABELING AND MEASUREMENT OF PRIMARY DAMAGE AND SECONDARY DEGENERATION

Primary damage of the optic nerve axons and their attached retinal ganglion cells (RGCs) were measured after the immediate
25 post-injury application of the fluorescent lipophilic dye 4-(4-(didecylamino)styryl)-n-methylpyridinium iodide (4-Di-10-Asp) (Molecular Probes Europe BV, Netherland) distal to the site of injury. Only axons that are intact are capable of transporting the dye back to their cell bodies; therefore, the number of
30 labeled cell bodies is a measure of the number of axons that survived the primary damage. Secondary degeneration was also measured by application of the dye distal to the injury site,

but 2 weeks after the primary lesion was inflicted. Application of the neurotracer dye distal to the site of the primary crush after 2 weeks ensures that only axons that survived both the primary damage and the secondary degeneration will be counted. This approach makes it possible to differentiate between neurons that are still functionally intact and neurons in which the axons are injured but the cell bodies are still viable, as only those neurons whose fibers are morphologically intact can take up dye applied distally to the site of injury and transport it to their cell bodies. Using this method, the number of labeled ganglion cells reliably reflects the number of still-functioning neurons. Labeling and measurement were done by exposing the right optic nerve for a second time, again without damaging the retinal blood supply. Complete axotomy was done 1-2 mm from the distal border of the injury site and solid crystals (0.2-0.4 mm in diameter) of 4-Di-10-Asp were deposited at the site of the newly formed axotomy. Uninjured optic nerves were similarly labeled at approximately the same distance from the globe. Five days after dye application, the rats were killed. The retina was detached from the eye, prepared as a flattened whole mount in 4% paraformaldehyde solution and examined for labeled ganglion cells by fluorescence microscopy. The percentage of RGCs surviving secondary degeneration was calculated using the following formula: (Number of spared neurons after secondary degeneration)/(Number of spared neurons after primary damage) x 100.

7.1.2 ELECTROPHYSIOLOGICAL RECORDINGS

Nerves were excised and their compound action potentials (CAPs) were recorded *in vitro* using a suction electrode experimental set-up (Yoles, E. et al., 1996, *J. Neurotrauma*, 13:49-57). At different times after injury and injection of T cells or PBS, rats were killed by

intraperitoneal injection of pentobarbitone (170 mg/kg) (CTS Chemical Industries, Israel). Both optic nerves were removed while still attached to the optic chiasma, and were immediately transferred to a vial containing a fresh salt solution consisting of 126 mM NaCl, 3 mM KCl, 1.25 mM NaH_2PO_4 , 26 mM NaHCO_3 , 2 mM MgSO_4 , 2 mM CaCl_2 and 10 mM D-glucose, aerated with 95% O_2 and 5% CO_2 at room temperature. After 1 hour, electrophysiological recordings were made. In the injured nerve, recordings were made in a segment distal to the injury site. This segment contains axons of viable retinal ganglion cells that have escaped both primary and secondary damage, as well as the distal stumps of non-viable retinal ganglion cells that have not yet undergone Wallerian degeneration. The nerve ends were connected to two suction Ag-AgCl electrodes immersed in the bathing solution at 37°C. A stimulating pulse was applied through the electrode, and the CAP was recorded by the distal electrode. A stimulator (SD9; Grass Medical Instruments, Quincy, Massachusetts) was used for supramaximal electrical stimulation at a rate of 1 pps to ensure stimulation of all propagating axons in the nerve. The measured signal was transmitted to a microelectrode AC amplifier (model 1800; A-M Systems, Everett, Washington). The data were processed using the LabView 2.1.1 data acquisition and management system (National Instruments, Austin, Texas). For each nerve, the difference between the peak amplitude and the mean plateau of eight CAPs was computed and was considered as proportional to the number of propagating axons in the optic nerve. The experiments were done by experimentors 'blinded' to sample identity. In each experiment the data were normalized relative to the mean CAP of the uninjured nerves from PBS-injected rats.

30

7.1.3 CLINICAL EVALUATION OF EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

Clinical disease was scored every 1 to 2 days according to the following neurological scale: 0, no abnormality; 1, tail atony; 2, hind limb paralysis; 3, paralysis extending to thoracic spine; 4, front limb paralysis; 5, moribund state.

7.2 RESULTS

7.2.1 NEUROPROTECTION BY AUTOIMMUNE anti-MBP T-CELLS

10 Morphological analyses were done to assess the effect of the T cells on the response of the nerve to injury, and specifically on secondary degeneration. Rats were injected intraperitoneally immediately after optic nerve injury with PBS or with 1×10^7 activated T cells of the various cell lines.

15 The degree of primary damage to the optic nerve axons and their attached RGCs was measured by injecting the dye 4-Di-10-Asp distal to the site of the lesion immediately after the injury. A time lapse of 2 weeks between a moderate crush injury and dye application is optimal for demonstrating the number of still-viable labeled neurons as a measure of secondary degeneration,

20 and as the response of secondary degeneration to treatment. Therefore, secondary degeneration was quantified by injecting the dye immediately or 2 weeks after the primary injury, and calculating the additional loss of RGCs between the first and the second injections of the dye. The percentage of RGCs that

25 had survived secondary degeneration was then calculated. The percentage of labeled RGCs (reflecting still-viable axons) was significantly greater in the retinas of the rats injected with anti-MBP T cells than in the retinas of the PBS-injected control rats (Fig. 2). In contrast, the percentage of labeled

30 RGCs in the retinas of the rats injected with anti-OVA or anti-p277 T cells was not significantly greater than that in the control retinas. Thus, although the three T-cell lines

accumulated at the site of injury, only the MBP-specific autoimmune T cells had a substantial effect in limiting the extent of secondary degeneration. Labeled RGCs of injured optic nerves of rats injected with PBS (Fig. 3A), with anti-p277 T cells (Fig. 3B) or with anti-MBP T cells were compared morphologically using micrographs (Fig. 3C).

7.2.2 CLINICAL SEVERITY OF EAE

Animals were injected i.p. with 10^7 T_{MBP} cells with or without concurrent optic nerve crush injury. The clinical course of the rats injected with the T_{MBP} cells was evaluated according to the neurological paralysis scale. Each group contained 5-9 rats. The functional autoimmunity of the injected anti-MBP T-cells was demonstrated by the development of transient EAE in the recipients of these cells. As can be seen in Fig. 4A, the course and severity of the EAE was not affected by the presence of the optic nerve crush injury.

7.2.3 SURVIVAL OF RGCs IN NON-INJURED NERVES

Animals were injected i.p. with 10^7 T_{MBP} cells or PBS. Two weeks later, 4-Di-10-Asp was applied to the optic nerves. After five days the retinas were excised and flat mounted. Labeled RGCs from five fields (located at approximately the same distance from the optic disk), in each retina were counted and their average number per area (mm^2) was calculated.

As can be seen in Fig. 4B, there is no difference in the number of surviving RGCs per area (mm^2) in non-injured optic nerves of rats injected with anti-MBP T-cells compared to in rats injected with PBS.

7.2.4 NEUROPROTECTION BY T-CELLS REACTIVE TO A CRYPTIC EPITOPE

To determine whether the neuroprotective effect of the anti-MBP T cells is correlated with their virulence, the effect

of T cells reactive to a 'cryptic' epitope of MBP, the peptide 51-70 (p51-70) was examined. 'Cryptic' epitopes activate specific T cells after an animal is immunized with the particular peptide, but not with the whole antigen (Mor, P. et al., 1995, *J. Immunol.* 155:3693-3699). The T-cell line reactive to the whole MBP and the T-cell line reactive to the cryptic epitope p51-70 were compared for the severity of the EAE they induced, and for their effects on secondary degeneration. In rats injected with the T-cell line reactive to the cryptic epitope, disease severity (as manifested by the maximal EAE score) was significantly lower than that in rats injected with the T-cell line reactive to the whole protein (Table 1). Whereas anti-MBP T cells caused clinical paralysis of the limbs, rats injected with the anti-p51-70 T cells developed only tail atony, not hind limb paralysis, and almost none showed weakness of the hind limbs. Despite this difference in EAE severity, the neuroprotective effect of the less virulent (anti-p51-70) T cells was similar to that of the more virulent (anti-MBP) T cells (Fig. 5). The percentage of RGCs surviving secondary degeneration in the retinas of rats injected with either of the lines was significantly higher than in the retinas of the PBS-injected rats. Thus, there was no correlation between the neuroprotective effect of the autoimmune T cells and their virulence. It is possible that the anti-p51-70 T cells encounter little antigen in the intact CNS, and therefore cause only mild EAE. Their target antigen may however become more available after injury, enabling these T cells to exert a neuroprotective effect.

TABLE 1. Anti-MBP and anti-p51-70 T cells vary in pathogenicity

T cell line	Clinical EAE	Mean max. score
Whole MBP	Moderate to severe	2.00 \pm 0.25
p51-70 of MBP	Mild	0.70 \pm 0.2

Immediately after optic nerve crush injury, Lewis rats were injected with activated anti-MBP T cells or anti-p51-70 T cells. The clinical course of EAE was evaluated according to the neurological paralysis scale. The mean maximal (max.) score \pm s.e.m. was calculated as the average maximal score of all the diseased rats in each group. The table is a summary of nine experiments. Each group contains five to ten rats. Statistical analysis showed a significant difference between the mean maximal score of rats injected with anti-MBP T cells and that of rats injected with anti-p51-70 T cells ($P=0.039$, Student's t-test).

7.2.5 ELECTROPHYSIOLOGICAL ACTIVITY

To confirm the neuroprotective effect of the anti-MBP T cells, electrophysiological studies were done. Immediately after optic nerve injury, the rats were injected intraperitoneally with PBS or with 1×10^7 activated anti-MBP or anti-OVA T cells. The optic nerves were excised 7, 11 or 14 days later and the compound action potentials (CAPs), a measure of nerve conduction, were recorded from the injured nerves. On day 14, the mean CAP amplitudes of the distal segments recorded from the injured nerves obtained from the PBS-injected control rats were 33% to 50% of those recorded from the rats injected with the anti-MBP T cells. (Fig. 6A, Table 2). As the distal segment of the injured nerve contains both axons that escaped the primary insult and injured axons that have not yet degenerated, the observed neuroprotective effect could reflect the rescue of spared neurons, or a delay of Wallerian degeneration of the injured neurons (which normally occurs in the distal stump), or both. No effect of the injected anti-MBP T cells on the mean CAP amplitudes of uninjured nerves was observed (Fig. 6B, Table 2). It is unlikely that the neuroprotective effect observed on day 14 could have been due to the regrowth of nerve fibers, as the time period was too short for this.

The strong neuroprotective effect of the anti-MBP T cells seen on day 14 was associated with a significantly decreased

CAP amplitude recorded on day 7 (Table 2). The anti-MBP T cells manifested no substantial effect on the uninjured nerve on day 7, indicating that the reduction in electrophysiological activity observed in the injured nerve on day 7 might reflect the larger number of T cells present at the injury site relative to the uninjured nerve (Fig. 1). The observed reduction in CAP amplitude in the injured nerve on day 7 reflected a transient reduction in conduction, which may have imposed a transient resting state in the injured nerve. This transient effect had not only disappeared, but was even reversed by day 14 (Table 2). Early signs of the neuroprotective effect could already be detected on day 11 in the rats injected with anti-MBP T cells (data not shown). In rats injected with anti-OVA T cells, no reduction in CAP amplitude on day 7 could be detected in either the injured or the uninjured nerves, and no neuroprotective effect was observed on day 14 (Table 2). Thus, it seems that the early reduction in CAP and the late neuroprotection shown specifically by the anti-MBP T cells are related.

TABLE 2. Transient reduction in electrophysiological activity of the injured optic nerve induced by anti-MBP T cells, followed by a neuroprotective effect

	Uninjured optic nerve		Injured optic nerve	
	Day 7	Day 14	Day 7	Day 14
Ratio (%)	89.9±9.4	101.2±22.7	63.8*±14.9	243.1**±70.8
T _{MPB} /PBS	(n=22)	(n=10)	(n=17)	(n=8)
Ratio (%)	109.7±13.2	92.5±12.6	125.5±24.4	107.3±38.9
T _{OVA} /PBS	(n=11)	(n=3)	(n=11)	(n=4)

Immediately after optic nerve injury, rats were injected with PBS or with activated anti-MBP or anti-OVA T cells. After 7 or 14 days, the CAPs of injured and uninjured nerves were recorded. Ratios were calculated for uninjured nerves as (mean CAP of uninjured nerves from T cell-injected rats/mean CAP of uninjured nerves from PBS-injected rats) x 100, or for injured nerves as (mean CAP of injured nerves from T cell-injected rats/mean CAP of injured nerves from PBS-injected rats) x 100. The P value was calculated by comparing the logarithms of the normalized CAP amplitudes of nerves from PBS-injected rats and rats injected with T cells, using the unpaired Student's t-test, *P<0.05; **P<0.01 n=sample size.

10

8. EXAMPLE: NEUROPROTECTIVE EFFECTS OF NS-SPECIFIC ANTIGEN

8.1 MATERIALS AND METHODS

Animals, crush injury of rat optic nerve, and retrograde labeling are described above in Sections 6 and 7. A peptide based on amino acids 35-55 of myelin/oligodendrocyte glycoprotein (MOG p35-55) was chemically synthesized at the Weizmann Institute, Israel.

8.1.1 INHIBITION OF SECONDARY DEGENERATION

Rats were injected intradermally in the footpads with MOG p35-55 (50 µg/animal) and IFA, or PBS ten days prior to optic nerve crush injury. Retinal ganglion cells were assessed two weeks after injury using retrograde labeling as described above. The number of RGCs in rats injected with PBS or MOG p35-55 was expressed as a percentage of the total number of neurons in rats injected with MOG p35-55 in the absence of crush injury.

8.2 RESULTS

As shown in Fig. 7, the number of labeled retinal ganglion cells (indicating viable axons) was about 12.5 fold greater in animals injected with MOG p35-55 compared to in animals receiving PBS.

9. **EXAMPLE: NEUROPROTECTIVE EFFECTS OF MBP**
ADMINISTERED ORALLY

9.1 **MATERIALS AND METHODS**

Animals, crush injury of rat optic nerve, and retrograde
5 labeling of RGCs are described above in Sections 6 and 7.

9.1.1 **INHIBITION OF SECONDARY DEGENERATION**

Bovine MBP (Sigma, Israel) (1 mg/dose) was administered to
rats by gavage using a blunt needle. MBP was administered 5
times, every third day, beginning 2 weeks prior to optic nerve
10 crush injury. The number of RGCs in treated animals was
expressed as a percentage of the total number of neurons in
animals subjected to optic nerve crush injury but which did not
receive MBP.

15 9.2 **RESULTS**

As shown in Fig. 8, the number of labeled RGCs was about
1.3 fold greater in animals treated with MBP compared to
untreated animals.

20 10. **DISCUSSION OF EXPERIMENTAL RESULTS**

The results of the experiments described in Sections 6 and
7 show that activated T-cells accumulate at a site of injury in
the CNS. Furthermore, the results also demonstrate that the
accumulation of T-cells at the site of injury is a non-specific
process, *i.e.*, T-cells which accumulated at the site of injury
25 included both T-cells which are activated by exposure to an
antigen present at the site of injury as well as T-cells which
are activated by an antigen not normally present in the
individual.

The results of experiments described in Section 7
30 demonstrate that the beneficial effects of T-cells in
ameliorating damage due to injury in the CNS are associated
with a NS-specific self-antigen as illustrated by MBP. More

specifically, the administration of non-recombinant T-cells which were activated by exposure to an antigen which can cause autoimmune disease (T_{MBP}), rather than aggravating the injury, led to a significant degree of protection from secondary
5 degeneration. Thus, activating T-cells by exposure to a fragment of a NS-specific antigen was beneficial in limiting the spread of injury in the CNS. The present findings show that secondary degeneration can be inhibited by the transfer into the individual of non-recombinant T-cells which recognize
10 a NS-specific self antigen which is present at a site of injury. The T-cells may recognize cryptic or non-pathogenic epitopes of NS-self antigens.

In addition, the studies described in Sections 8 and 9 show that activation of T-cells by administering an immunogenic antigen (e.g. MBP) or immunogenic epitope of an antigen (e.g.
15 MOG p35-55), may be used for preventing or inhibiting secondary CNS degeneration following injury.

The present invention is not to be limited in scope by the exemplified embodiments, which are intended as illustrations of
20 single aspects of the invention. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and accompanying drawings. Such modifications are intended to fall within the scope of the
25 appended claims.

All publications cited herein are incorporated by reference in their entirety.

30

WHAT IS CLAIMED IS:

1. A method for preventing or inhibiting axonal degeneration in the central nervous system or peripheral nervous
5 system comprising administering to a human in need thereof:

- (a) non-recombinant, NS-specific antiseif activated T-cells;
 - (b) a NS-specific antigen;
 - (c) a peptide derived from a NS-specific antigen;
 - 10 (d) a nucleotide sequence encoding a NS-specific antigen;
 - (e) a nucleotide sequence encoding a peptide derived from a NS-specific antigen; or
 - (f) any combination of (a)-(e),
- to ameliorate the effects of injury or disease.

15

2. A method for promoting nerve regeneration in the central nervous system or peripheral nervous system comprising administering to a human in need thereof:

- (a) non-recombinant, NS-specific antiseif activated T-cells;
 - 20 (b) a NS-specific antigen;
 - (c) a peptide derived from a NS-specific antigen;
 - (d) a nucleotide sequence encoding a NS-specific antigen;
 - 25 (e) a nucleotide sequence encoding a peptide derived from a NS-specific antigen; or
 - (f) any combination of (a)-(e),
- to ameliorate the effects of injury or disease.

30

3. The method according to claim 1 or 2 in which said injury comprises blunt trauma, penetrating trauma, hemorrhagic stroke, ischemic stroke, or damages caused by

surgery.

4. The method of claim 1 or 2 in which said disease
is Diabetic neuropathy, senile dementia, Alzheimer's disease,
5 Parkinson's Disease, facial nerve (Bell's) palsy, glaucoma,
Huntington's chorea, amyotrophic lateral sclerosis, non-
arteritic optic neuropathy, or vitamin deficiency.

5. The method of claim 1 or 2 in which said disease
10 is not an autoimmune disease or a neoplasm.

6. The method of claim 1 or 2 in which said peptide
derived from a NS-specific antigen is an immunogenic epitope or
a cryptic epitope.

15

7. The method according to claims 1 or 2 in which
said NS-specific antigen is administered intravenously,
intraperitoneally, intramuscularly, subcutaneously, orally,
intranasally, vaginally, rectally, intraocularly, intrathecally,
20 intradermally, or buccally.

8. The method according to claim 1(a), 1(c), 1(d),
1(e), 2(a), 2(c), 2(d), or 2(e), further comprising
administering to a human in need thereof a NS-specific antigen.

25

9. The method according to claim 8 in which said NS-
specific antigen is administered before or after administration
of the composition according to claim 1(a), 1(c), 1(d), 1(e),
2(a), 2(c) or 2(e).

30

10. The method according to claim 8 in which said NS-
specific antigen is administered concurrently with

administration of the composition according to claim 1(a), 1(c), 1(d), 1(e), 2(a), 2(c) or 2(e).

11. The method according to claim 1 or 2 in which
5 said T-cells are attenuated.

12. The method according to claim 1 or 2 in which
said T-cells are autologous or allogeneic.

10 13. The method according to claim 1 or 2 in which the
NS-specific antigen or peptide derived therefrom is myelin basic
protein, myelin oligodendrocyte glycoprotein, proteolipid
protein, myelin-associated glycoprotein, S-100, β -amyloid, Thy-
1, P0, or P2.

15 14. The method according to claim 1d or 2d in which
the nucleotide sequence is depicted in Fig. 9, Fig. 10, Fig.
11(A-F), Fig. 12, Fig. 13, or Fig. 14.

20 15. The method according to claim 1 or 2 in which the
NS-specific antigen comprises the amino acid sequence of Fig.
15, Fig. 16, or Fig. 17.

25

30

ABSTRACT OF THE INVENTION

The present invention discloses compositions and methods for the treatment of injury or disease of the nervous system CNS. In a particular embodiment, the invention provides methods
5 of treatment using non-recombinant activated antiself T-cells that recognize an antigen of the NS or a peptide derived therefrom or a derivative thereof to promote nerve regeneration or to prevent or inhibit axonal degeneration within the NS. The invention also provides methods of treatment using a NS-specific
10 antigen or peptide derived therefrom or a derivative thereof or a nucleotide sequence encoding said antigen or peptide to promote nerve regeneration or to prevent or inhibit axonal degeneration in NS, i.e., the CNS and/or PNS. The NS-specific antiself activated T-cells may be administered alone or in
15 combination with NS-specific antigen or peptide derived therefrom or a derivative thereof or a nucleotide sequence encoding said antigen or peptide or any combination thereof.

20

25

30

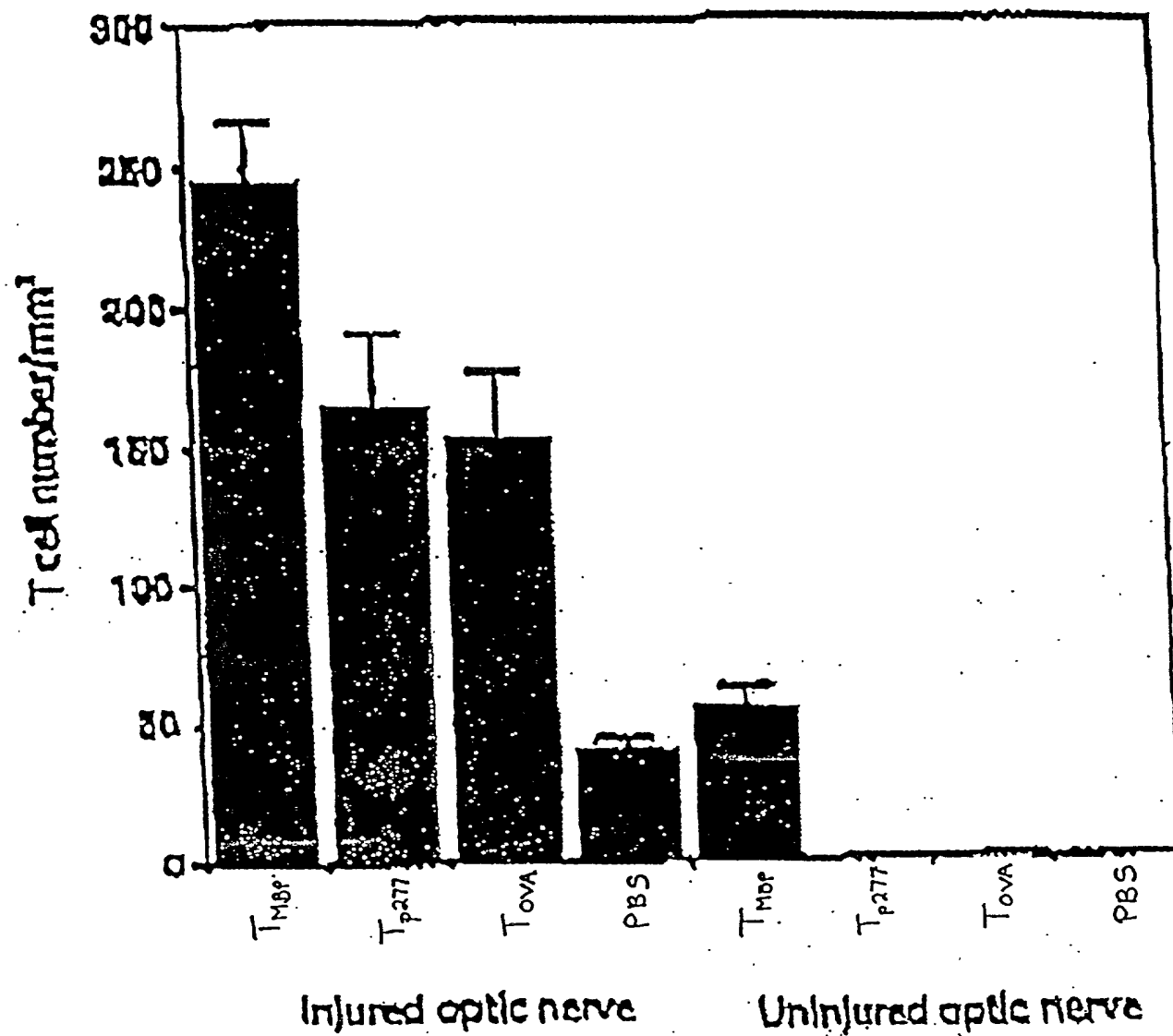


FIG. 1

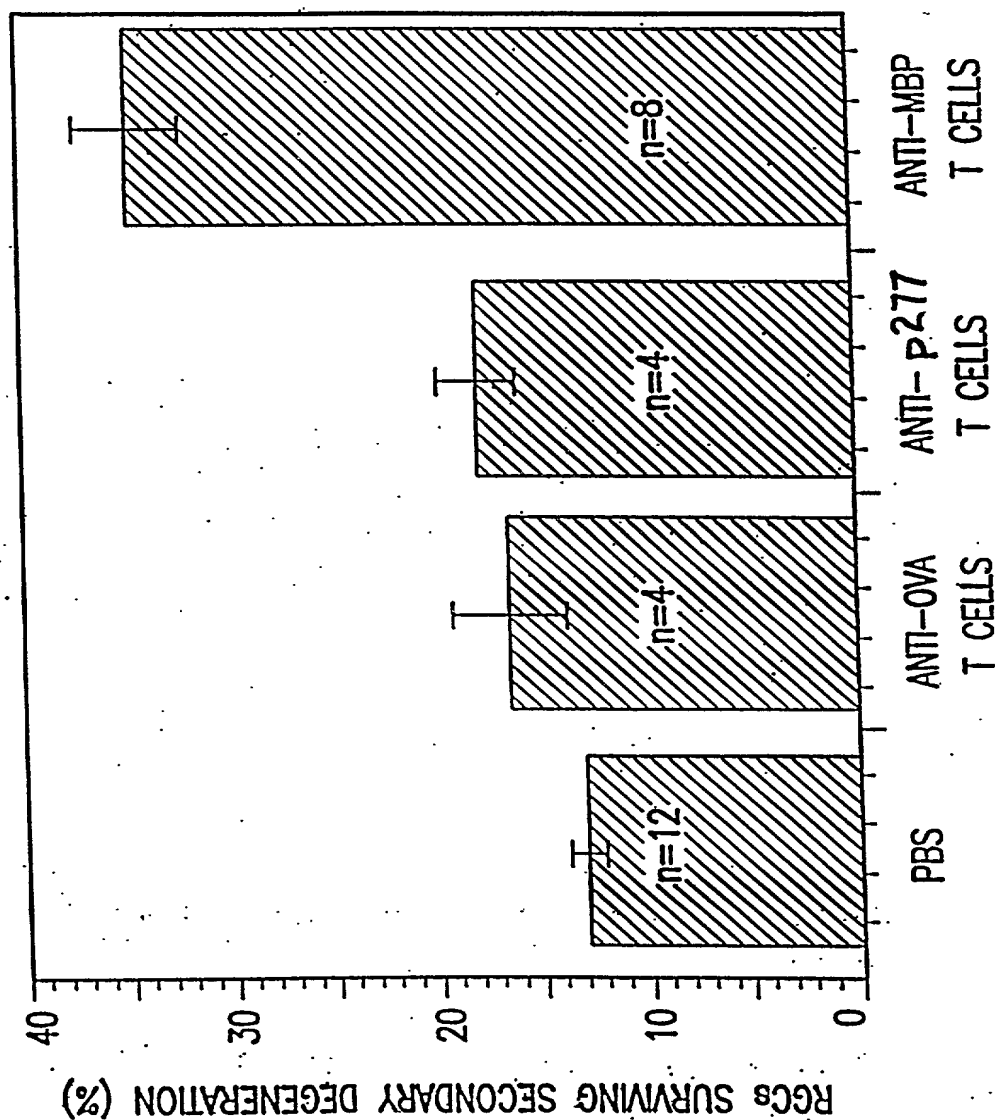


FIG. 2



FIG. 3A

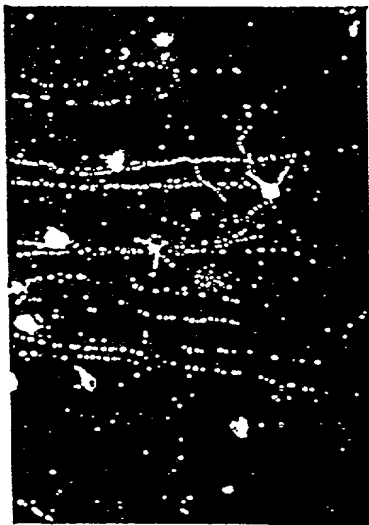


FIG. 3B

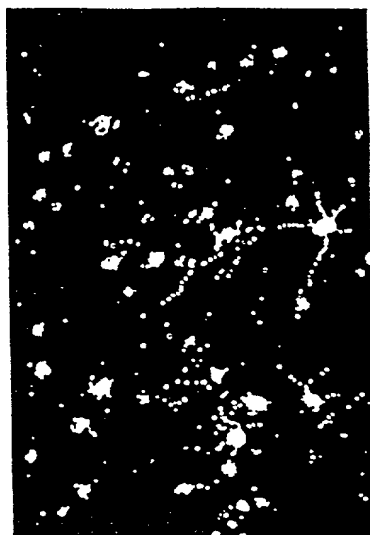


FIG. 3C

160 μ m

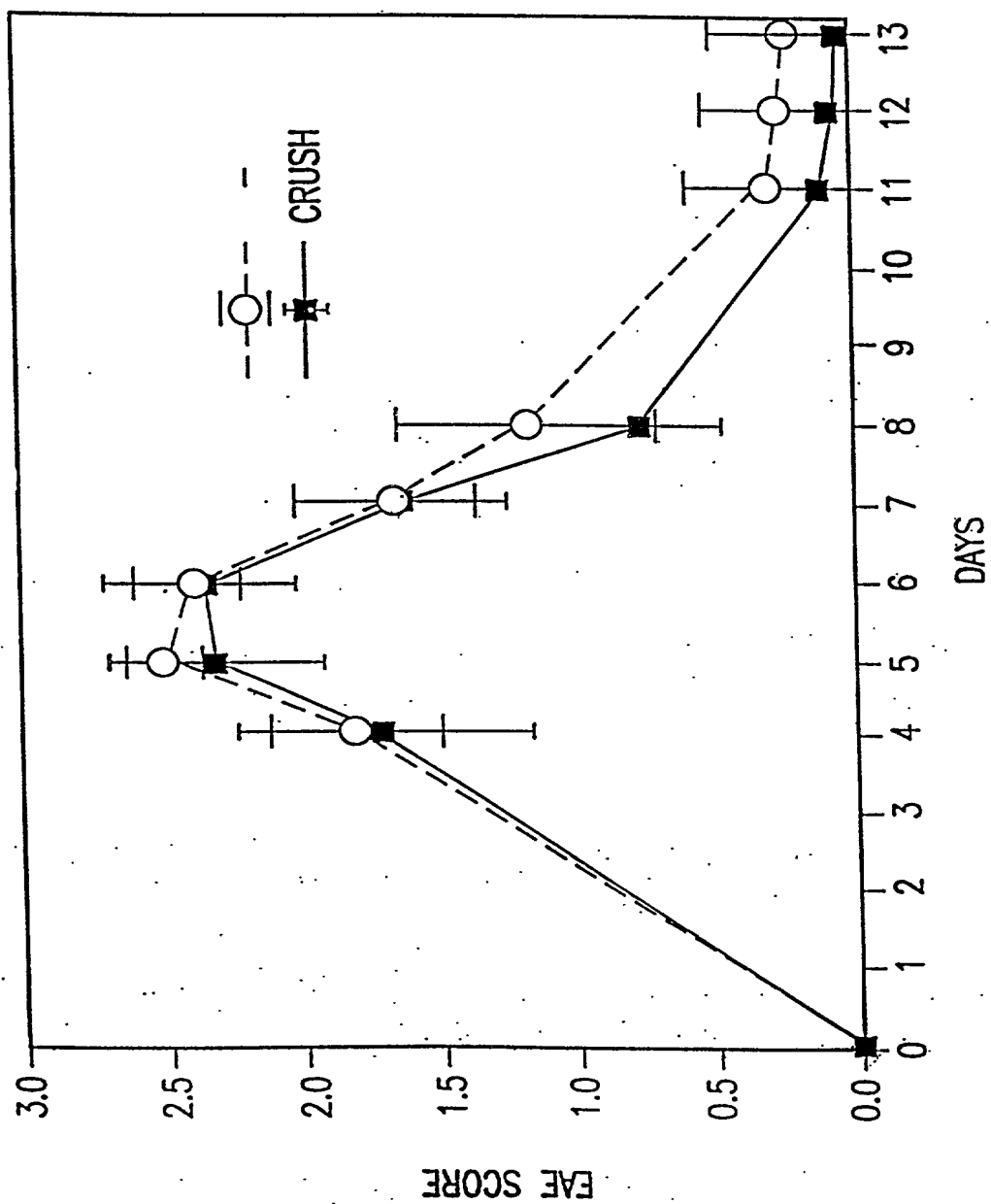


FIG. 4A

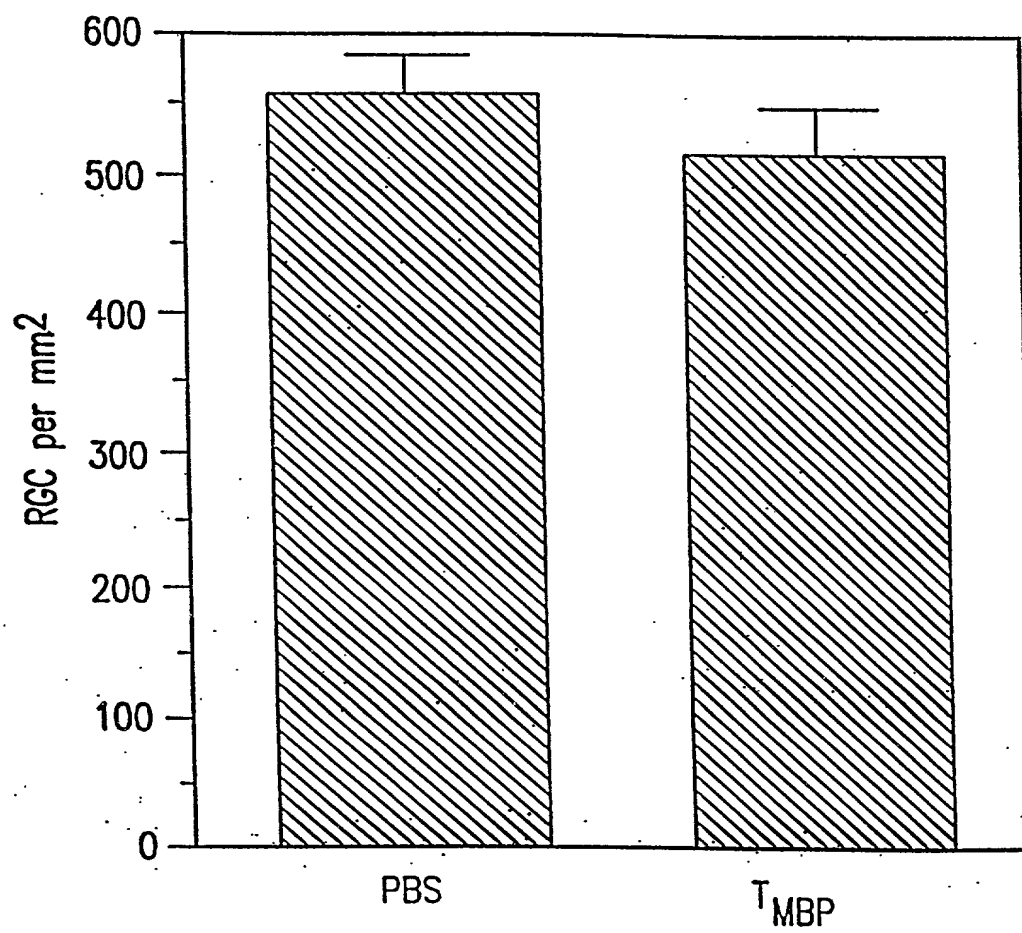


FIG. 4B

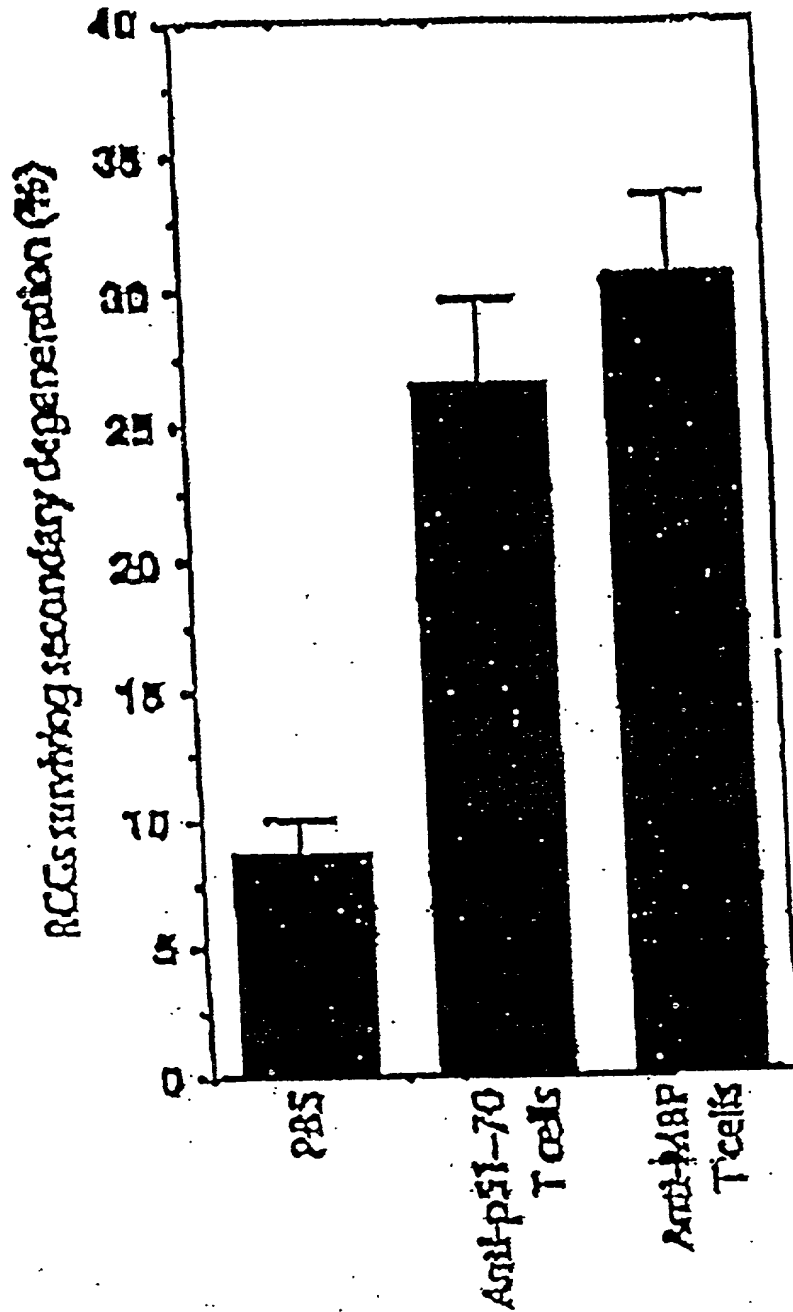


FIG. 5

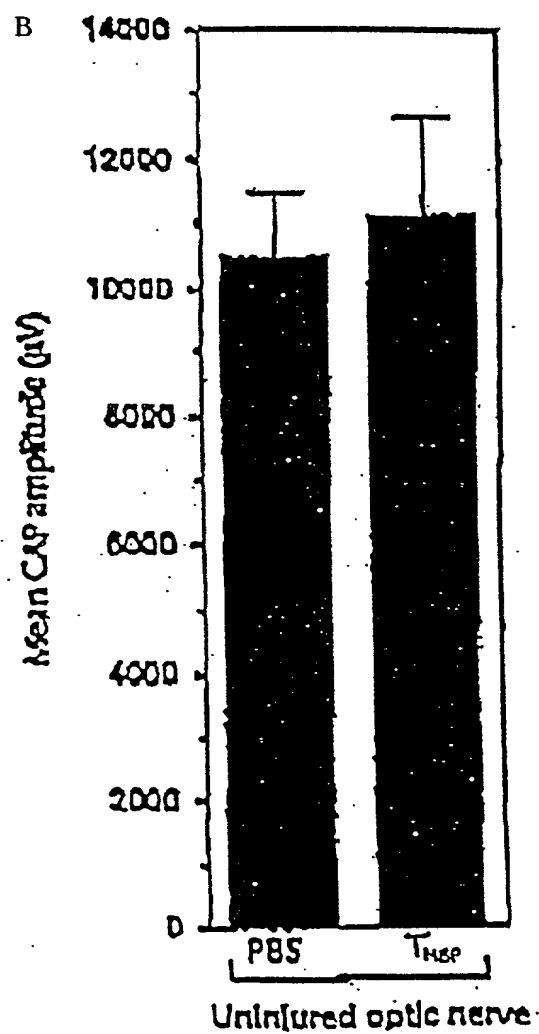
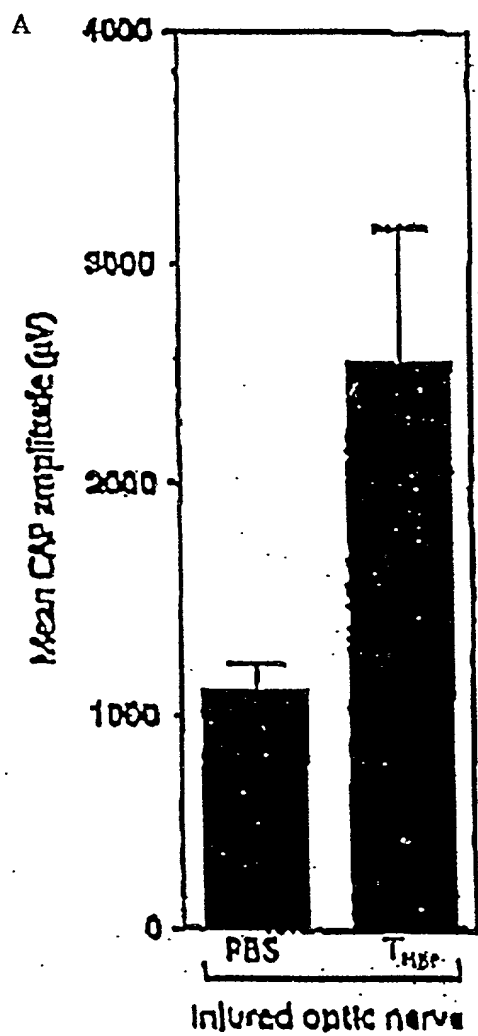


FIG. 6

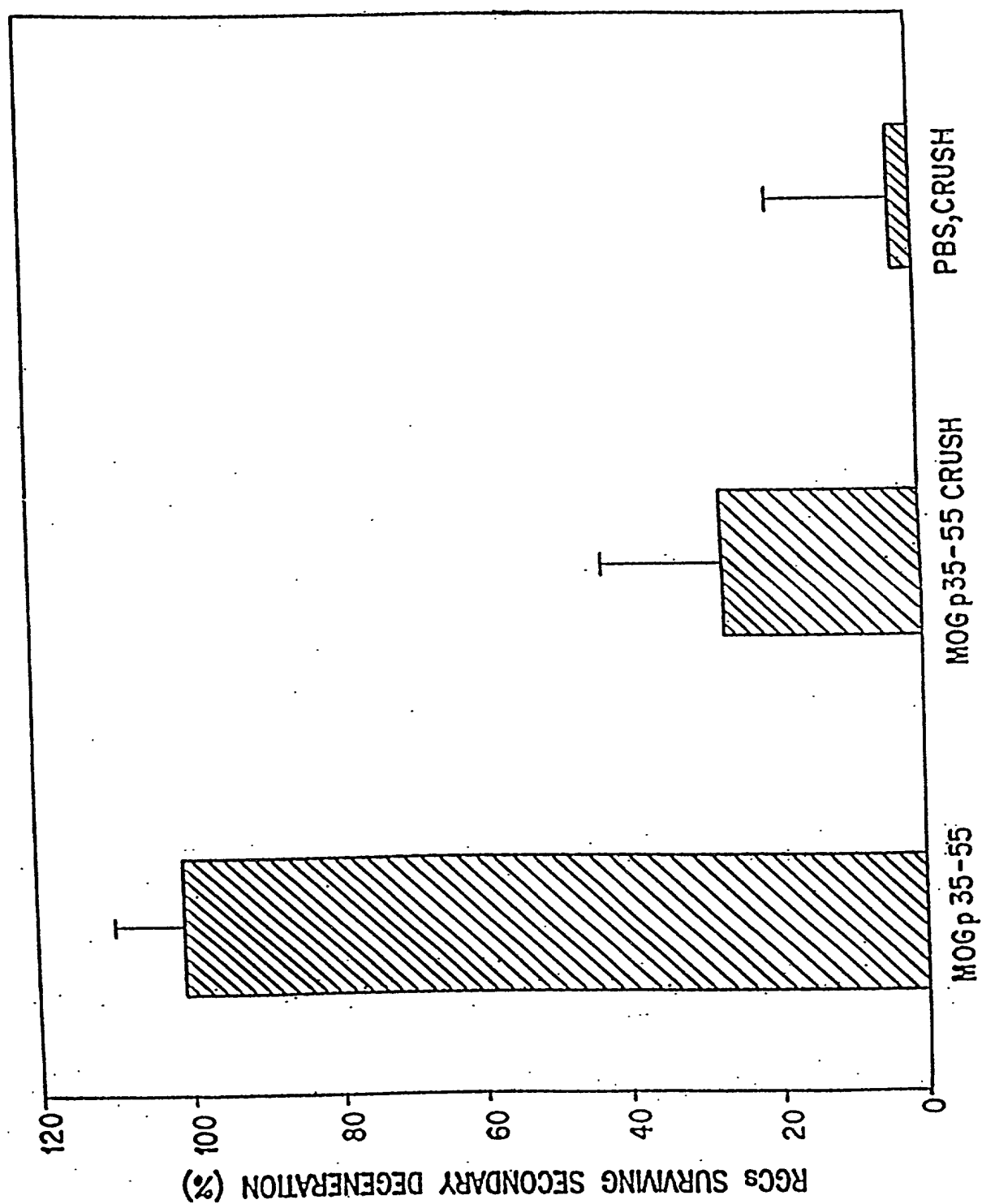


FIG. 7

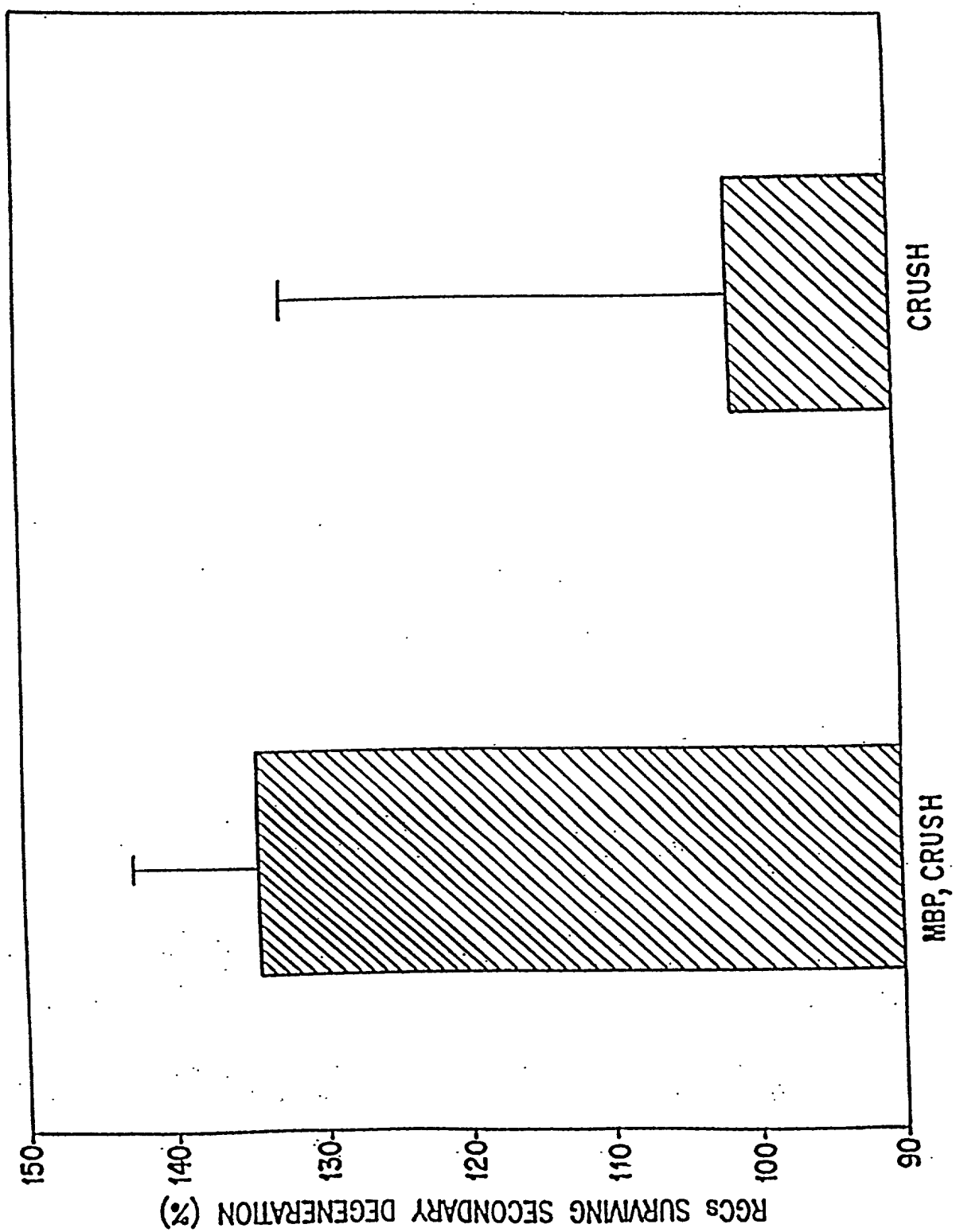


FIG. 8

1 ccaagaagat cccacagcag cttccgaagg cctggatgtg atggcatcac agaagagacc
61 ctacacagcga cacggatcca agtacttggc cacagcaagt accatggacc atgcccggca
121 tggcttcctc ccaaggcaca gagacacggg catccttgac tccatcgggc gcttctttag
181 cggtgacagg ggtgcgcca agcggggctc tggcaaggac tcacacacaa gaactacca
241 ctacggctcc ctgccccaga agtcgcagag gaccaagat gaaaaccag tagtccactt
301 cttcaagaac attgtgacac ctcgtaacc ccctccatcc caaggaaagg ggagaggcct
361 gtccctcagc agatttagct ggggaggaag agacagccgc tctggatctc ccatggcaag
421 acgctgagag cctccctgct cagccttccc gaatcctgcc ctgggcttct taatataact
481 gccttaaagc ttttaattcta cttgcaccaa atagctagtt agagcagacc ctctcttaat
541 cccgtggggc tgtgaacgcg gcgggccagc ccacggcacc ctgactggct aaaactgttt
601 gtcccttttt at

FIG. 9

1 gaaaacagtg cagccaccto cgagagcctg gatgtgatgg cgtcacagaa gagaccttcc
 61 cagaggcacg gatccaagta cctggccaca gcaagtacca tggaccatgc caggcatggc
 121 ttctctccaa ggcacagaga cacgggcato cttgactcca tcgggcgctt ctttggcggc
 181 gacaggggtg cgccaaagcg gggctctggc aaggactcac accacccggc aagaactgct
 241 cactatggct ccctgcccc aaggtcacac ggccggaccc aagatgaaaa ccccgtagtc
 301 cacttcttca agaacattgt gacgcctcgc acaccacccc cgtcgcaggg aaaggggaga
 361 ggactgtccc tgagcagatt tagctggggg gccgaaggcc agagaccagg atttggctac
 421 ggaggcagag cgtccgacta taaatcggct cacaagggat tcaaggagat cgatgccag
 481 ggcacgcttt ccaaaatttt taagctggga ggaagagata gtcgctctgg atcaccatg
 541 gctagacgct gaaaacccac ctggttccgg aatcctgtcc tcagcttctt aatataactg
 601 ccttaaaact ttaatccccc ttgcccctgt tacctaatta gacagatga cccctcccct
 661 aatgcctgcg gaggttgtga cgtagtagg tcaggccacg gcagcctacc ggcattttcc
 721 ggccaacagt taaatgagaa catgaaaaca gaaaacggtt aaaactgtcc ctttctgtgt
 781 gaagatcacg ttcttcccc cgcaatgtgc cccagacgc acgtgggtct tcagggggcc
 841 aggtgcacag acgtccctcc acgttcaccc ctccaccctt ggactttctt ttccgctgg
 901 ctgggcaccc ttgcgctttt gctggctact gccatggagg cacacagctg cagagacaga
 961 gaggacgtgg gcggcagaga ggactgttga catccaagct toctttgttt ttttttctg
 1021 tccttctctc acctcctaaa gtagacttca ttttctctaa caggattaga cagtcaagga
 1081 gtggcttact acatgtggga gcttttttgt atgtgacatg cgggctgggc agctgttaga
 1141 gtccaacgtg gggcagcaea gagagggggc cacctcccca ggccgtggct gccacacac
 1201 cccaattagc tgaattcgcg tgtggcagag ggaggaaaag gaggcaaacg tgggctgggc
 1261 aatggcctca cataggaaac agggctcttc tggagatttg gtgatggaga tgtcaagcag
 1321 gtggcctctg gacgtcacog ttgccctgca tgggtggccc agagcagcct ctatgaacaa
 1381 cctcgtttcc aaaccacagc ccacagccgg agagtccagg aagacttgcg cactcagagc
 1441 agaagggtag gagtccctca gacagcctcg cagccgcgcc agtcgcccac agacactggc
 1501 tgtgaccggg cgtgctggca gcggcagtg acagtggcca gactaaccac tccctgagaa
 1561 gataaccggc tcattcactt cctcccagaa gacgcgtggt agcgagtagg cacaggcgtg
 1621 cacctgctcc cgaattactc accgagacac acgggctgag cagacggccc ctgtgatgga
 1681 gacaaagagc tcttctgacc atatccttct taacaccgcg tggcatctcc tttcgcgct
 1741 cctccctaa cctactgacc caccttttga ttttagcgca cctgtgattg ataggccttc
 1801 caaagagtcc cacgtggca tcacctccc cgaggacgga gatgaggagt agtcagcgtg
 1861 atgccaaaac gcgtcttctt aatccaattc taattctgaa tgtttcgtgt gggcttaata
 1921 ccatgtctat taatatatag cctcgatgat gagagagtta caaagaacaa aactccagac
 1981 acaaacctcc aaatttttca gcagaagcac tctgcgtcgc tgagctgagg tcggctctgc
 2041 gatccatacg tggccgcacc cacacagcac gtgctgtgac gatggctgaa cggaaagtgt
 2101 acactgttcc tgaatattga aataaaacaa taaactttt

FIG. 10

A.

1	taatatctag	ggktttgact	ctgacccgtg	ttggggctct	cacttcatgg	cttctcacgc
61	ttgtgctgca	tatcccacac	caattagacc	caaggatcag	ttggaagttt	ccaggacatc
121	ttcattttat	ttccaccctc	aatccacatt	tccagatgtc	tctgcagcaa	agcgaaattc
181	caggcaagcc	ttagggaaaa	aaggaaaaac	aaagaaaatg	aaacaattgg	cagtgaaggg
241	cagaaagaga	agatggagcc	cttagagaag	ggagtatccc	tgagttaggtg	gggaaaaggg
301	gaggagaagg	ggaggaggag	aggaggagga	aagcaggcct	gtccctttaa	gggggttggc
361	tgtcaatcag	aaagcccttt	tcattgcagg	agaagaggac	aaagatactc	agagagaaaa
421	agtaaaagac	cgaagaagga	ggctggagag	accaggatcc	ttccagctga	acaaagtcag
481	ccacaaagca	gactagccag	ccggctacaa	ttggagtcag	agtcccaaag	acatgggtaa
541	gtttcaaaaa	cttttagcatt	gaagattcaa	gaggacacag	g	

B.

1	ctgetttcag	agcctgtgac	ttcttgtgtg	cctctcctgt	ttctcagcaa	catggcatag
61	ggcctgggat	accagggtctg	gggatctcag	ggactcttag	cactttaaga	cacatgtgtt
121	cccaggccct	ggtgtgttcc	tctagtcca	gaaagatgtt	tcatgctttg	ctgactttgt
181	ataaagtctg	ttttagctg	ttttgacaga	atctcagcgt	ataactgagg	gtggggacat
241	tagccaagct	gcattatagg	aggacaaaac	tgccatacaa	agtgtccaaa	atcattaagc
301	ctgcattttt	attattggga	gtaatatcaa	acctcctatt	ttccaatttt	catttcttgt
361	cctgtgctag	ctccatcctg	tttggactgc	tcctcccata	tgtaaaactaa	gaagaatcaa
421	gcattctttg	caacaaatâc	acacgatgct	caaaaatgtc	caggagcatc	caatttccaa
481	agtttctctc	acctggaatg	ctcttcatgc	taaaatcctg	tctgacaata	ccagcatctc
541	tggcctgcac	tcatcccttc	ctggaactcc	aagtgcattt	accctctgtt	accacttact
601	tggctgcctg	aattgttagt	tgaaaatatt	aggtctactt	agctaattct	tcctcaggaa
661	attaaagact	cccataggc	agagtctgtg	tcttttctct	cttcatatcc	cgtataacac
721	ccagcataat	gctgggcata	tagtgagtat	tcataaaata	gttgatgaat	gactaaaata
781	agcaagcaaa	caaacagact	agaacaataa	gaaagaaggg	actggatttc	ataatctctc
841	tggcttgcta	tttgaattgc	tgaattatta	ttatttatta	aatatttttt	aaattctggc
901	aataaaaggt	aaggatttat	tttctttctt	tctttttttt	tttcttgaga	cagagtctcg
961	ctcttactgc	ccaggctgga	gtacaatggc	gcaatcttgg	ctcacggcaa	cctccgcctc
1021	ctcctggggt	taacagattc	tcctgtctca	gcctcctgag	tagctgggat	tacaggcata
1081	cgcccatgcc	cggctaattt	ttgtattttt	agtagagacg	gggttttgcc	atgttggcca
1141	ggctggctct	gaactcctga	cctcatgtga	tcacactgcc	tcagcctccc	aaagtgtctg
1201	gattacaggc	atgcgccacc	gtgcccggcc	aaagatttat	tttcaagaat	gaaacaaagt
1261	aaggattctg	ggtcaatctc	acatgctgaa	agccaaaacc	tctagccgct	cctgcttttt
1321	gacttcggag	tgcccactat	ctccgagcct	gtgagcacag	ggcctggcag	aggggtttga
1381	gtggcatgag	ctacctaactg	gatgtgcctg	actgtttccc	cttcttcttc	cccaggcttg
1441	ttagagtgtc	gtgcaagatg	tctggtaggg	gccccctttg	cttccctggg	ggccactgga
1501	ttgtgtttct	ttgggtggc	actgttctgt	ggctgtggac	atgaagccct	cactggcaca
1561	gaaaagctaa	ttgagacct	tttctccaaa	aactaccaag	actatgagta	tctcatcaat
1621	gtgtaagtac	ctgccctccc	acacagaccc	atcttttttt	tcctctctc	catcctggag
1681	atagagaact	cttcagtacc	ttagtaacta	gcaggggact	ggggtggagc	cagaccggat
1741	tcccgagtct	tcctctgtg	ca			

FIGS. 11A-B

C. 1 ctagaaaatc cctagccttg ttaaggtgct cgctctggtg tatacctcac ttatgtcggg
61 aaagaagcca ggtcttcaat taataagatt ccctggtctc gtttgtctac ctgttaatgc
121 aggatccatg ccttccagta tgtcatctat ggaactgcct ctttcttctt cctttatggg
181 gccctcctgc tggctgaggg cttctacacc accggcgag tcaggcagat ctttggcgac
241 tacaagacca ccatctgcgg caagggcctg agcgcaacgg taacaggggg ccagaagggg
301 aggggttcca gaggccaaaca tcaagctcat tctttggagc ggggtgtgtca ttgtttggga
361 aaatggctag gacatccga caaggtgatc atcctcagga ttttgtggca ataacaaggg
421 gtgggggaaa attggggcgg agtctgtggc ctctcctcca cccaaggctg ggtcctctct
481 aggggcctgg catttgagtg aggaagcgat ggctgcagcc gaacgagaag gtcaggaaga
541 acgtggtgcc cagctggctt agcctcacct ttcaaagggt ccctaagcaa atttcttctc
601 aaaacagaaa gcatgagttt tgtgggatgc tttgtacaat cagaccattt ctaagccatc
661 tgttggtatc cctttgttcc cttcctagta ggtaccacaa gagtggatct aactggacaa
721 gagtctaaaa tgctgctcat gtgattgaga cttgggcacc tgagctraga gggaggatgg
781 ataataaaaa ttaataata actccaaggt aaatttacia tgttctg

D. 1 gatcctctc attcttcccc taccatttcc cccaccctc cgttatactg gggccagtta
61 tctagtagat actgccaatt acccttggca gaggtgccct gctcactaat tttatttggg
121 ggagmgccct ggaacctggt tttaatgtct ggcacacgcc acttccagga tctcccagtt
181 tgtgtttcta catctgcagg ctgatgctga tttctaacca acccatgtca atcatttttag
241 tttgtgggca tcacctatgc cctgaccgtt gtgtggctcc tgggttttgc ctgctctgct
301 gtgcctgtgt acatttactt caacacctgg accacctgcc agtctattgc cttccccagc
361 aagacctctg ccagtatagg cagtctctgt gctgatgcca gaatgtatgg tgagttaggg
421 tacgggtgct ttggctctcc taccactat ggaagcacta tatatttggg tattttctta
481 gtgtaaggag ggtggtgatt atgagaaaaa tataagatga tgaatgattg ggtcttagtt
541 tattaatcct tccctactga aaccagagag gtttcttccc ccggaaggga acttgggaagt
601 ggtgggagtt ttcttggcca ttcacattgg cctactctag ttgactgctg ttcacaaccc
661 caaagcagca catttcaata acaaacacaa ggttdsacca ctgttcaata ccaccttctc
721 ttttttgtaa acctgtagaa aagaggatcc taattgttgg tagmatccaa mtttacagcc
781 aggataatta gagatggaag aagggtctctg ggggaaagtc tccatgtggc cccgtaactc
841 cataaagctt accctgcttg ctttttgtgt cttacttagg tgttctccca tgggaatgctt
901 tccctggcaa ggtttgtggc tccaaccttc tgtccatctg caaaacagct gaggtgagtg
961 ggttatttgg gttattttac aaggagtag ctaataccat acaaattaca cccatggcct
1021 tcaattttaa ggactgaaag ttcccttttg ctggattttg aattagccga ttgccttcta
1081 caacatgttg gctaagtgtg cctgagccaa tgagcataga aggtaaaaca cctcttttct

E. 1 aattagcaca cagaaaggat atccaacaca tacaaagctg tnntcatgga ctacactgga
61 gcatattact gctgttgcaa gaaacatttc ttcttctct tttcattttc ctgcagttcc
121 aaatgacctt ccacctgttt attgctgcat ttgtgggggc tgcagctaca ctggtttccc
181 tgggtgagttg actttgaatg atcttggcaa gtaaataggc ctgagatagt tgtgggtaca
241 gctattctga aaggcaagaa ggtagactgc ttccatcctt gaaatgctgg aggga

FIGS. 11C-E

F.

1	aattctatat	actatcacta	tggctccact	ttggatactc	tccagtggat	ttagttaactc
61	atatggaaat	acctgggagg	acctcctaac	attattagaa	ttgttatgat	tataatacaa
121	ygctatgtcc	caggtcttgc	tgatagtgtc	acagtgccct	gtgaatgtag	tgtgtctcatt
181	gtgcagatta	aaaacctaag	gcactgaagg	gtgaagtgat	ttatctgaag	ttattttata
241	aagcagtgat	cagacaasct	gagctcacag	aactccctgg	cccctactgc	tgagggttcc
301	atacagagtc	aagtaatttc	tcaccttgta	aaacgaattg	attcattaac	cagggggagag
361	ctctactgca	tgatgtggct	gtgtgtctac	agcaagcacc	ctatgactct	aagtcactcg
421	gacatattga	tgtggcaaaag	cccaaattatt	gttcacttcc	ctgaggaaaa	ctcagtgcta
481	gatcaaacag	aggtgtggaa	taaatcttta	tgatttgatt	ctctgggcct	gggccatgag
541	acccatgatg	cctcagagac	atcggacttc	cagtcaagtg	tatatggaga	aagccaagcc
601	tgggatgtac	tgctttttgc	agagcatggg	ttttccctt	atthagttat	gattttat
661	ctacccttcc	tcattcccaa	agggatttga	ggagggagtg	ctttcttttc	tactctcatt
721	cacattctct	cttctgttcc	ctacagctca	ccttcatgat	tgtctgccact	tacaactttg
781	cogtccttaa	actcatgggc	cgaggcacca	agttctgac	ccccgtagaa	atcccccttt
841	ctctaatagc	gaggctctaa	ccacacagcc	tacaatgctg	cgtctcccat	cttaactctt
901	tgcctttgcc	accaactggc	cctcttctta	cttgatgagt	gtaacaagaa	aggagagtct
961	tgcagtgatt	aaggtctctc	tttggactct	ccctctttat	gtacctcttt	tagtcatttt
1021	gcttcatagc	tggttccctgc	tagaaatggg	aaatgcctaa	taatatgact	tcccaactgc
1081	aagtcacaaa	ggaatggagg	ctctaattga	attttcaagc	atctcctgag	gatcagaaaag
1141	taatttcttc	tcaaagggtta	cttccactga	tggaaacaaa	gtggaaggaa	agatgctcag
1201	gtacagagaa	ggaatgtctt	tggtctctct	gccatctata	ggggccaaat	atattctctt
1261	tgggtgtacaa	aatggaattc	attctgctgc	tctctattac	actgaagata	gaagaaaaaa
1321	gaatgtcaga	aaaacaataa	gagcgtttgc	ccaaatctgc	ctattgcagc	tgggagaagg
1381	gggtcaaagc	aaggatcttt	caccacagac	aagagagcac	tgaccccgat	ggcgatggac
1441	tactgaagcc	ctaactcagc	caaccttact	tacagcataa	gggagcgtag	aatctgtgta
1501	gacgaagggg	gcatctggcc	ttacacctcg	ttagggaaga	gaaacagggg	cttgtcagca
1561	tcttctcact	cccttctcct	tgataacagc	taccatgaca	accctgtggg	ttccaaggag
1621	ctgagaatag	aaggaaacta	gcttacatga	gaacagactg	gcctgaggag	cagcagttgc
1681	tgggtggctaa	tggtgtaacc	tgagatggcc	ctctggtaga	cacaggatag	ataactcttt
1741	ggatagcatg	tcttttttct	tgtaaattag	ttgtgtactc	tggcctctgt	catatcttca
1801	caatgggtgt	catttcatgg	ggtattatcc	attcagtcac	cgtaggtgat	ttgaagggtct
1861	tgatttggtt	tagaatgatg	cacatttcat	gtattccagt	ttgtttatta	cttatttggg
1921	gttgcatcag	aaatgtctgg	agaataattc	tttgattatg	actgtttttt	aaactaggaa
1981	aattggacat	taagcatcac	aaatgatatt	aaaaattggc	tagttgaatc	tattgggatt
2041	ttctacaagt	attctgcctt	tcagaaaaca	gatttggtga	atttgaatct	caatttgagt
2101	aatctgatcg	ttctttctag	ctaattgaaa	atgattttac	ttagcaatgt	tatcttgggtg
2161	tgttaagagt	taggtttaac	ataaagggtta	ttttctcctg	atatagatca	cataacagaa
2221	tgcaccagtc	atcagctatt	cagttggtaa	gcttccagtc	atcagctatt	cagttggtaa
2281	gcttcccagg	aaaaaggaca	ggcagaaaga	gtttgagacc	tgaatagctc	ccagatttca
2341	gtcttttaat	gtttttgtta	actttgggtt	aaaaaaaaaa	aaagtctgat	tggttttta
2401	tgaaggaaag	atttgtaacta	cagttctttt	gttgtaaaga	gttggtgtgt	tcttttcccc
2461	caaagtggtt	tcagcaatat	ttaaggagat	gtaagagctt	tacaaaaaga	cacttgatac
2521	ttgttttcaa	accagtatac	aagataagct	tccaggctgc	atagaaggag	gagagggaaa
2581	atgttttgta	agaaaccaat	caagataaag	gacagtgaag	taatccgtac	cttgtgtttt
2641	gttttgattt	aataacataa	caaataacca	acccttccct	gaaaacctca	catgcataca
2701	tacacatata	tacacaca	aagagagtta	atcactgaa	agtgttccct	catttctgat
2761	atagaattgc	aattttaaca	cacataaagg	ataaactttt	agaaacttat	cttacaaagt
2821	gtattttata	aaattaaaga	aaataaaatt	aagaatgttc	tcaatcaaac	atcgtgtcct
2881	ttgagtgaat	tgttctattt	gacttcacaa	tagaaactta	ataatcgtac	cttctcaaga

FIG. 11F

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1  atggaaatgt tctgtatttg tgttgtctga tgagataacc actaactgta gtgctattga
61  gcatttgaaa catggctagt gtaatcaatg aaccaaattt ttaattttat ttaattgtaa
121 ttaatttttaa gtggccacat gcagggagtg actgctgcat tggacagcac ggctctaaat
181 tgagcctttt ttccttattt ggtgaggcat acttgcccta agattgggaa gtctattttt
241 ggaacctgct accaatgctg gtctcacact tgcaattctc agctgagcca agaggtgaga
301 gaaaggctcat tttccattcc aagatctcac tctcccctgt gacactgagg aaactggcaa
361 gtgatgtgaa ggctggagag cgtgtcctgt atgctggctc tgtcccttct gcctgtgttg
421 actgacatag ttagttgctg cccttgctgg tctcccttcc tccaaccttg cctctctgag
481 cacacctgac attcatctca tgacttcctt aaaaacattc tttgggaaca agaaactaac
541 aaatcccaag tgacctatca catatacaaa catacagggc agagtttgga ttcgcggtag
601 aagaaaggga ggttagacat taagaagaat ggtctggtga tgacagttgt gagataatag
661 aaacaggaaa aagaaatcta agttttcttt ctttttttaa gaaccaataa taatttctct
721 cttttgacta gtcagtaggg ctgggggtgga ttggaggaag cttacatatt ccatgaacaa
781 gcctcttctt aaggctcctgt aagtgtactt gcccactga ttagccctta gaagaccctt
841 caaaggtttg atctccagga gggagtgggg gaggaaagcc ctgtaccagg cagcctctgc
901 tccattgctc tgggggggtg ggggaagaca accctggtca tcccctcagt ctgtagccct
961 tttgtgtgag tgcctggcaa ggggtgacgt gggctgtttc tgcgggcaca gctgcagcaa
1021 ttaccggagt ggaggcaggg cccaggcagc actgccctcc aagatcttcc cttgggcttt
1081 tcagcagtaa ggggacatgc accccaaggg cctccacttg gcctgacctt gctgcggggg
1141 ctctctgtcc ccaggaacag tagagatggc aagcttatcg agacctctc tgcccagctg
1201 cctctgctcc ttcctcctcc tccctcctcc ccaagtgtct tccagctatg caggtaagac
1261 atgttttttt tccctgccctg gggagaccct gaaaacagaa aggctagttt cctgggggtt
1321 agctccttca aacatcctca agttgggtata ttatctttct aaaacataga cctactgaca
1381 tgctccctt cctcagaaac cttccgtggg tggttcttac agccttcaag atggagtcca
1441 gactcttttt tttttttggg acagagtctc cctctgttgc tcaggctgga gtgcagtggc
1501 atgatctcgg ctactgcaa cctcagcctc cctggttcaa gcgattctcc tgacttggcc
1561 tcccaagtag cggagactac aggcgcctgc caccacaccc agctaaattt gttcttttct
1621 ttcttttttt ttttttttgg gatttttagga cagacggggt ttcacatggt ggccaggatg
1681 gtctcgatct cttagactgc tgatccgccc gcctcagctt cccaaagtac tgggattatg
1741 ggctgtagcc actgcactag gcctaatttt tttattttta gtagagatgg ggtttcacca
1801 tggtagccag gctggtctgg aacctctgac ctcaagtggg ctgcccctct cagcctccca
1861 aagttctgag attacaggca tgagccattg cgtctgacct agactcctta atgtgactaa
1921 ctccaggctt tccttggaact acttcttact tgtctttcca gctttgtctt ttcacctctc
1981 caattgagat aaaataataa caacctcttg gatttctcat caggattaca tgaatgaga
2041 tatgtaacat gcttagcagt gcctgtccat agtaaatctc aataaatggt tgtggaatta
2101 taatatcttg tcatgtttga gactttgtct tgcataatca ggcaccagta ggtttttata
2161 aaggaacccg tctgtcacgt gcagaggaga aataaacaga aagtttccca tcctcaggga
2221 gccacctgac tgacagaggc acagtgcata cactctccag gtctagggga gaaagcagcc
2281 ttattttctta gtagctcaga atctgacttg agaaacacat ccacatagaa aaaaacaagg
2341 aactttttct ggtcagggct cgggacccac agtgaggtgg aagatacagg ggaaggaaga
2401 gggaaataga gccatcccca gggtggaaga tctcagaaga gaatttggga aacaaggtat
2461 gaacaaggac tgaatagtga gaagtgtagg agagacagct aaagtagatg gagtgtcaaa
2521 accaaaacct ctaagggttag aataggcagc aatttggcca agtcctaaca gggaggccca
2581 taggaggatt caacctcaag atgctgtgcc acattccaag agggaaacct aaggctgggc
2641 tgaagagtca gagatggcta cagctggcaa aaagatgggc agatgctgag aggagatgat
2701 tgctaaaaat ttctgtccag gacattcaca gtatctctat aaccagagto tttttgtctg
2761 ttgttgttct caagaaggaa acttgaggcc ggggtgtggtg gtttatgccc ataatcccag
2821 cgctttgggg ccaaggcagg cggtacacct gaggtcagga gttogagacc agcctggcca
2881 acagtgtgaa acctcatctt tactaaaaat acaaaaatta gctggatgag gcggtagggtg
2941 cctgtaatgc cagctactcg ggaggctgag gcaggagaat cacttgaacc tgggaggcgg
3001 aggttgcagg gaggcggagg ttgcagttag ccaagattgc accactgcac tocagcctgg

```

FIG. 12

3061 ggcacagaga gtaagactgt ctcaaaaaat aaatgaataa ataaaaagga agaagaagaa
 3121 gaagaacaat tgcaatcctc cctggctcta gaatgtcatt taaaagtcga gtgtcttctt
 3181 ccttcctgt tttgaagcag ccctttctcat gacaggcttg ctggccaagg ttccctctga
 3241 ccttaaatct cttccttttg gtgtcttgga cagggcagtt cagagtata ggaccaagac
 3301 accctatccg ggctctgggc ggggatgaag tggaattgcc atgtcgata tctcctggga
 3361 agaacgctac aggcattggag gtggggtggt accgcccccc cttctctagg gtgggtctatc
 3421 tctacagaaa tggcaaggac caagatggag accaggcacc tgaatatcgg gcccgagacag
 3481 agctgctgaa agatgctatt ggtgagggaa aggtgactct caggatccgg aatgtaaggt
 3541 tctcagatga aggaggtttc acctgcttct tccgagatca ttcttaccaa gaggaggcag
 3601 caatggaatt gaaagtagaa ggtgagtagt gccatataat attaggtatt aactgttggg
 3661 tggccaagaa caattattct ctcaactgag atgagatccc tcaacccaaa catctcagtc
 3721 ctgggaatga tttccataaa aatgtacaca tcaataaaca gaaactcatg cttagggatg
 3781 tctgttgcat cattattcag agtagcaagg aaattgggat caaaatcaat gcctttgagt
 3841 aggttaagtga cagaatgaac aatggtagcc atactgtgaa tattatgcag ggattaaaaa
 3901 gattattttta gcactaggcc agatggtttg gggggctcct ctaagggtatt attgagtgat
 3961 aagagcaagc tgctgtagga tacaaaaaca aaaacaaaac cctagggcatt ggtggtttgc
 4021 ctgcgagcta ctcaggaggc tgagacggga ggctggcttg agcccagggg ttgcagtta
 4081 cagtgaagcta tgattgcacc actgcactcc aaccgggtg acagagcaaa gaccttcacc
 4141 cccactccct acccgtctct aaaaaaaaaca aaaacaaaaa caaaaaaacc cttgggcccc
 4201 gcgccgtggc tcacgcctgt aatcccagca ctgtgggagg ccgaggtggg cagatcacaa
 4261 ggtcaggaga tcgagaccat cctggctaaa acggtgaaac cccgtctcta ctaaaaatac
 4321 aaaaaaaaaa aaaaaattta gccaggcatg gtagcaggcg cctgtagtcc cagctactcg
 4381 ggaggctgag gcaggagaat ggcgtgaacc cggaagcggg ggttgcaatg agccaaatc
 4441 cttccactgc actccagcat gggggacaca gcgagactcc gtctcaaaaa aaaaaaaaaa
 4501 accctgtatt tgtgagcgca cacacacaca cacacacaca cacacctgtg cttggtccta
 4561 gtgaataagc aagtaaataa aatgtctaaa tataattata gaaaggagat gtcacctttt
 4621 ggctgtacct ccactatttc attctgcaga attgcagaat ttcttttttt ttctctttct
 4681 ttctttttct tttttttttg acacagagtc tcgctctgta acccaggctg gactgcaatg
 4741 gcgcctccg cctcctgggt tcaagtatt ctcctgcctc agcctccgga gtagctggga
 4801 ttacaggtgc ccaccaccac acccagctaa tttttgtatt tttagtagag acagggtttc
 4861 accaggttgt caaggttggt ctcaaaactc tgacctcagg tgatccactc gcctcagact
 4921 cccaaagtgc tgggattaca ggcatgagcc atggtgcccg gcctcagaat ttcatittca
 4981 acatgttttg catgatgggt gattttgagg aatatttttt gctctatcgc aggatgatta
 5041 agatgtggac aaggtgaagc cgatggaggg ggagctttga aagttacttg ctatttaatt
 5101 gaggaactaa actgctttga gagcctgggg gtcagatcct ctgccttttc ctctcccca
 5161 cctgcagtgc aaacatcaga caattgatca ctattgtatc ttggaggtgg gactgaccat
 5221 tgcagtgtcg ggaccagaag atggcattgt atgtggaaca acaaagcact atttctagag
 5281 actgcctgca gggatatgga aatagcttta tgtgtctcag aatgttcttc atacagctgt
 5341 ttttatttgg gaaattctac ttgccgaaaa gtttgatagt gagaccctct ccagtttgca
 5401 gattttttct cttcctgtct aacaacttcc tagctcagta actgcctctc ccaacaaact
 5461 cctcagttt caccacacca aaaaaggaag acaagccggt tgcgggtggc cacacctata
 5521 atcccaaaac tttgggaggc cgaggcgggt ggatccacct gaggtcggga gttcgagact
 5581 agcctgacca acatggagaa accctgtctc tactaaaaac acaaaattag cctggcgtgg
 5641 tggcgcatte ctgtaatccc agctgggagg ctgaggcagg agaatcgctt gaaccccgga
 5701 ggccggaggt gcagtgaagc aagatcgctc cattacactc cagtctgggc aagaaaagtg
 5761 gaactccatc tccaaaaaaa aaaaaaaa aacaaggaag acaaaaagaa aagcagctaa
 5821 agactttgac tcaggggaga aagttctctt ttgggttgct atccacattc caacctctg
 5881 ttcccaactc ttgctctgca tgctaaagaa actgttttac aagtaaataa gggacgcttt
 5941 gtctaggctt tggagccagg aagttgagac aaatttagga atgagatgaa gtaatggtat
 6001 tattgcaagt ctgaggtgta actacctctg ctctttctct gaagagtttc taatttctct
 6061 tgtttactta tttttttctt gtcatttttg ggattttatt actagttgtc tctaactcct

FIG. 12 (cont.)

6121 tcttttaaatt cttcattatg aaacataaaa acaaatgcc a ggcgcggcag ctcacgcctg
 6181 taatcccagc actttgggag gccgaagcgg gcagatcacc cgggtcagga gttcgagacc
 6241 agcctgatca acatggagaa acccgcgtct tactaaaaaa tacaaaatta gctaggcggtg
 6301 gtggcacatg ccagtaatcc cagctacttg agagactgag gcaggagaat cgcttgaacc
 6361 gggaggcaga gggtgcggtg agccaagatc gcgccattgc actccagcct gggcaacaag
 6421 agcaaaactc tgtctcaaaa aaaaaaaacc acatacaaac cagagataat attataatga
 6481 gcctccaagt gcctaccacc ttgctgcagc acttgtcaat ccagggacca cccacctcac
 6541 cggctcccca ctcattacca ccctccccta ctcaattact gaggtaaatc ctaggcagca
 6601 tgatcatttc ttttttttct ttttatttat tttgagacag gatctgtctc tgtcaccag
 6661 gctggagtgt agtggcatat ctctgtcac tgcagcctct gcctcccggt cagaagccat
 6721 cctcccacct cagcctacat agtagctggg accacaggca cacaccacca cacactgcta
 6781 atgttttgta ttttttgtag agactgggtt ttaccatgtt gatcaggctg gtctcaaact
 6841 cctaggctca agcaatcctc ccacctcggc ctcccaaagt gctagaatta caggcgcgag
 6901 ccaactgcacc cagcgaagaa cactttttta aaaataaata ggcggggcgc ggtggctcac
 6961 acctgtaatc ccagtaactt gggagcccaa ggagggcgaa tcatgaggtc aagagattga
 7021 gaccatccta agtaacatgg tgaacccca tttctactac aaatacaaaa acaaaattag
 7081 cctggcgtgg tggcaggcgc ctgtagtccc agctacttgg gagctgaggc aggagaatgg
 7141 agtgaaccgg ggaggcggag cttgcagtga gctgagatca tgccactgca ctccccctg
 7201 gggcaacaga gtgagactcc caaaaaaaa aaaaaaagcc cccctcccc acacacaata
 7261 atataaataa ataaataacc acaatactat tatcacatct taaaaactca acaaaaattt
 7321 cttaatatca tcaaataacc agtttgtgtt caaatttttc tgattgtttc ataaatatac
 7381 tcttacagtt ggtttctttt agcgagattc aaatgagacc cacctgttga cctttgccct
 7441 taggggtttc cagggtctga attttgttga cgacattccc atgttgctat gtaatacggg
 7501 cctccatgcc ctgtgttttt ctgtaaactg atagatgtgg aggtgcaatg acatttgtgt
 7561 ttgatttact ttggcaataa tagttcatca gtgatactct atacttcttg ttgctttaca
 7621 tccggaggct gataatgtct gcttttctct cttttctaat tttttgtgaa aggaaaaatg
 7681 tgggggggtt ggagaaaaaa acccttaagt acatactgc taaatcacat tgctacaggt
 7741 aacttccatt aagaacttga aagtaaaagg agctgcattt tcccctaggg aacacaatga
 7801 tagacaggag ccttagtcta cagcttgaag gattgttaatt atacctaaagc aaccctcctg
 7861 gaccagttta atgttattag ctgtgatgta tccctacctt tgatgtcatt atccttactt
 7921 agctccctta aagcagagat caagatgaaa agggcttcag ctgcagcatg gcacatggag
 7981 attagagtgg ggcttttgga tgctgaggag cagacctaga atgggaaata gatgggagcc
 8041 acagaagtga aggtccccct cctcatttgc tcaacctact ccacatctcc aggtctgcac
 8101 atctgttcag ttactgaatc ctgtgtaagc taccttcttt ttcttttttc ttttatttat
 8161 ttattttatt tttttttgag atggagtttt gctcttgtaa ccagggttg tgcaattctc ctccctcagc
 8221 tgcaatctcg gctcactgca cctccaact ccagggttca tgcaattctc ctccctcagc
 8281 cttccaagta gctgggatta caggctgcac caccatgtct ggctaatttt tgaaaaatca
 8341 gtagagagag ggtttcacca tgttgcccaa gccggtctcg aactcctgac ctcaagtgat
 8401 ccaccacact tggcctccca aaatgctggg attacagggt tgagccacca tgcccgtgt
 8461 aaactacctt cttaaaagct ctagaagagg gcttttaacc ttttggtgtg tgtcatgcac
 8521 ctcccgcaag ctgatgaagt tgatagacct atctcagaat tttttttttt tttttgagac
 8581 agtgtctcac tctgtcacc caggattggt gcagtggcac gatcatgggt cattgcagcc
 8641 tccacctccc aggtcaagt gatcctcctg actcagcctc ttgaatagct gagaccacag
 8701 gcttgtgtca ccattgcccag gtaattttta attttttttc gtagaggcag ggtctcacat
 8761 tatgttgccc agtctggcct cgagaactcc tgggctcaag caatcttctt gccttgggct
 8821 cccaaagtgg tgggattaca ggggagagcc accacacct a ggcaggagga tgtttttaaa
 8881 acaccaataa aaacatttat acccaaatac agttatccaa atattaaatt aacaagagtt
 8941 agggtgacct tattaattag tgaatttccc aaatagtaat gaacataagt gatagtttga
 9001 gatttctgtg acttttctaa tgtgacgtga aaatatttgt gatttttttt tttctttttt
 9061 ttttttgaga tggagtttct ctcttgttgc ccaggctgga gtgcaatggc aagatctcgg
 9121 ctcacctcaa cctccgcctc ctgggttcaa gcgattctcc tgctcagcc tcttgagtag

FIG. 12 (cont.)

9181 ctgggattac aggactgtgc caccacgtcc agctaatttt gtatttttag tagaaacagg
 9241 gttttcccat gttggtcagg ctgggtcttga actcccaacc tcaggcgatc cgccgcctc
 9301 ggcctcccaa agtgctggga ttacaggtgt gagccaccgc acctggccaa tatttgtgat
 9361 ttttattgac gacaaagtca aagggtctct tcatattatt gtggtgtatc gcctacaagc
 9421 ataattaaaa taaacactaa atttcagttt aaagtttact gaaaataaat atgtattttt
 9481 tattccctat ttaagctttg aatccccctga ctccctatac cattaccact gtcctagttc
 9541 aggttcatgt tgttttttac ttttaattggt atcacagtct cttaacattt ctccctatgt
 9601 tctccagtc tgtaggtgct aaatctgacg tggtcacttc tcagcttgga atccttcagt
 9661 gcaccaccac agccttgaaac tacatatattg aaatacatat ttattttcag taaactttaa
 9721 actgaaattt agtgtttatt ttaattatgc ttgtaggcga tacaccacaa taatatgaag
 9781 agaacctttg actttgtcgt caataaaaag tcccttgagg ggacttcaga tgtaagttcc
 9841 ttagtctctc gttaaaactc cccaggtctg acccaatata caatcttgac tttaaaccac
 9901 ttgtcattct aaatcactag ctttctctgg aaaaaaagc catttttctc tcagggctaa
 9961 gctcagggac caattctgtg tcacctctct tgaatcctga tgatattcac ttctttattt
 10021 gacctgattt attgggcccc agacaccatg ctgagtgttg gggattcagc tctggacaat
 10081 gtcaaagtgc agtccctgct ttcagatcct ttctactggg tgagccctgg agtgcctggt
 10141 ctccctcgcg tgetgctgt gctcctcctg cagatcactc ttggcctcgt ctccctctgc
 10201 ctgcagtaca gactgagagg tacagggcag aggggtgggt gatcaggatc ctttctttaa
 10261 atgagctggc ttcttgaggc tacaccactt aacatgtatt tgtgagtac ttctgggttc
 10321 agaagttctt ctactattg agtgataaag aaaaaaata actccatgat gaaagagttt
 10381 tacatcttac ggaatgcttt catatgaata atcggaccta gcatttccct atgagctaac
 10441 tatgccatat agtaacccca ttttacagag gatacaactg aggccaggag tagttcagtg
 10501 acttactcaa accgatataa cttataagtg gtagagctga ggcctctgta tcataacctag
 10561 cagctccatg caacttgagg gagtgtgagc ttcgaaagca gacaggtcta ggctattagg
 10621 agttttgaat aaagatactg aagtgaagt ctctaccaca cagtaggcgt tcgaaaattg
 10681 tttcctcttt ctccattcaa cactgaggac tcaggttcag ctgctgatga agctcctctt
 10741 ttttgccatg agctttcatt ctgagccttc tcctcctacc aagtgtctcc ccaatgccag
 10801 agcaggaaga gtcttcactc ctcccaatgc cccacctccc atttggtact aagaggagag
 10861 gagaaagtag caaggagggt atggggaatg ttctggggga atgggtgttg gtgcgatcaa
 10921 caacaaagtc ctttctctca ccttgaattc atcccagatg cctgctgttt tacttcttcc
 10981 acacaaaaaa aggccttcag ccctcatggc tgagcagaaa gaatctgaat gttagagtea
 11041 ggcagcctgg gtttgaattc catctcaggt actgaactct atagcaaaat tcttagattc
 11101 tccaagcttc agttgccttg tctgtcaaat agagaaaaca tccttcgtcc taaattgtag
 11161 ggaggattaa agtcatgcaa agtgccctact acaaatccag tcacaaagta gctagctact
 11221 cactaaatgt tcagctcctc cctcctcatt cagatgggaa gtggcttttag ataaacaaag
 11281 tggcaacgca gtgggctgga gcagctctgt gaactgagaa tccaagaaaa ggggcgaaga
 11341 gcagctggga tgtattggat gcttgtgctg gcttggagca ttgctcacat tctttattcg
 11401 ctattgtatc tagactatag cttagaaaag agccgcaacc attggcttta aatccagtgc
 11461 tcttcctact ctctgaggt tgtttccagg ctgcagagaa atagcctgca caaggggccc
 11521 aggcgctggg tgtgggaggg tccccaccga gagccagaa atgcaggaaac taaaatgttg
 11581 cctttttcta ttttaggaaa acttcagagc gagataggtg agttccagtc atcgtttctc
 11641 ccaattcttg ccttttggtt ttttggcata acggaaatgg tcccattctt ggaccgtctc
 11701 tccctctcaa taccctgttt tccctcagtt ttccctttct ctacagtggg tgtgtcgtgc
 11761 ctagaacaag ttttaagtaa ttaataaca aagactcagg ataaaaggat cctttttgga
 11821 gtgcctact aaatccattt ccatttgttt ctctttcaga gaatctccac cggacttttg
 11881 gtaagtccg gcatgtctag gccctcccag gtcaacttgg tatttcactc tagttccagt
 11941 cacctggggg aacaaggacc cctggctcct ggttgagtc ctccctctct tctctttct
 12001 ttctttaaat aagaagtcatt ttgcatttag gattggtaaa atcataataa aaatactcat
 12061 gtactgtttt tatgtgccag gcactattct aactacttta caaaaacgtt atcttattct
 12121 gtttaactcc ttatgcacat gatctctctt ttcaggaaat ccaaaacaga ggtaaataga
 12181 tcgtttacac gtaaacctga tgtctggttg gggaggtgaa acaaacagaa acaagacaca

FIG. 12 (cont.)

12241 actgtatcac ctgtacttat atttctgctt tacaaactca ggatgtttcc atgagtacag
 12301 aacatgacta atcagagaag acctcataga ggaatagaaa agccaccaag cccactagg
 12361 aattgacccc tcaaggacat ggtttctagc ctttttggtc actgcagatt gcccaatgcc
 12421 taaagataat ggcaacagaa gagcacccaa atatttggtt gataaatggt gcagacacta
 12481 gaagggtgtca ttagggcaca gatggtacct tctctgagca aacttccttc acagctcctc
 12541 ctcccagagg tgtagggtgac tctactcttg tcacctggca cacagagtgc tatcgtacga
 12601 tttaggaaat tagaccagtg tgtggaccac acacacacac atctttacac acccaaagag
 12661 gaggaatagt atctttgttt tggaggactt gactatgaaa ggtcttaact cctttttgta
 12721 ccatgaatct ctctggcact ccagtgaagt ctaaaggacc cctttgcaga atgtttttaa
 12781 atatacacat aaaatagaac acataggatt gcaaaaacaa tcattgtact aaaatacagt
 12841 tatcaaccga taatcacatt tgtgatatag taacataaat gtttcttttt tttttttttg
 12901 gaggcagagt ttggctcttg tcaccagggc tggagtgcga tggcgcgatc taggtcact
 12961 gaaacctctg cctcccgggt tcaagcgatt ctacgcctcc tgagttagctg ggattacagg
 13021 tgcccgccac cacacccagc taatttttgt atttttagta gagactaggt ttcaccaggt
 13081 tggccaggct ggcctcgaac tctgacctc aggtgatcca cctgccttgg cctcccaaag
 13141 tgctgggatt acgggcatga gccaccgtgc ccggccataa atatttcttt agccaaagta
 13201 atacattaag taatgtagca gcaagtctaa taacctgtaa tttctttctt tctttctttc
 13261 tttctttttt tttgagatga agtttttttg agatggagtg caatggcaca atctcggctc
 13321 actgcaacct ccacctcctg ggttcaagcg attctcctgc ctacgcctcc caagttgctg
 13381 gaactacagg cgcatgccac catgcccagc taatttttgt atttttagta gagacggggg
 13441 ttcaccatgt tggccaggct ggtcttgaa cctgacctc aggtgatctg cctgccttgg
 13501 ccttccaaag tgctgggatt acaggcatga gccaccaggc ccagcccaat aacctttaat
 13561 ttcaacatac taataaacat aaacagtatt tcaagatttc tgcaataact ctaatgggaa
 13621 tgaaaacatc tgtggcttcc attggttaatt aagtcacagg tactgctcat attgtgggta
 13681 gttgtaaaat gttttggttt gttttgtttt ttccaagact tgggggaatg ggtgttggtg
 13741 ggatcaacaa gagtcttgct ctgtggccca ggctggagtg caggggcagg atcttggtc
 13801 actgcaacct ccgctccca ggttcaagcg attctcctgc ctacgcctcc tgagttagctg
 13861 gcattacagg catgtgccac cacgcccagc taatttttac atttttagta gagatggggg
 13921 ttcaccatgt tggcctggct ggtctgaaac tcttgccctc atgatccacc cgtctcggac
 13981 tcccagagtg ttgggattac aggcattgag caccacacct ggcagttggt acatttttaa
 14041 tgaaagaaaa tgttaaatcc agttattgaa aataaggagg cagtactttt ctcatccaag
 14101 ttcattggact ttctgaattt tgtccccaga gtcttttgtt gttctaggac cccagggtta
 14161 ggaacccaaa aagacagggt ggtggggcat gagggggaac acatgttaat cctgtttgt
 14221 tctggtgaac aattcagatc cccactttct gaggggtgcc tgctggaaga taacctgtt
 14281 tgtaattgtg ccggttcttg gacccttggg tgccttgatc atctgctaca actggctaca
 14341 tgaagacta gcagggtgcag tggctgggca gcaggcaaga ccaccaaata gtgggggacc
 14401 aagtcagctc tgaatgggaa gccaaaagag aatagaacca ggactcaaga ttaggggagc
 14461 tgggatttcc ttattcctct gtcccatgac ccaacccag gctcttctga gaaactgtga
 14521 agagaaccac ttactggatc tgtgggatcc cccagtggaa agggcagtggt gggctactcc
 14581 aaatgtccat agggaggatg tggggaagggt gctattcacc ttccactaat cacatatttg
 14641 tttctttttg ttttcagggc aattccttga agagctacgt aagttctctt ctctctgta
 14701 taagcagaga ataaaaagcc aggaaaggga gacagaagca acaagaggaa gaggcgggct
 14761 attgagggat cacattccca gaggaaagga ggagctggag agcctgggtg gagggaagac
 14821 tctcctggg aggttagagg caaagaagcc agctgttaga gacacattta caggtggcag
 14881 agaagctgga ggcactccta tctgccacct ctgcacattc tcttctactg cccctaagca
 14941 ggaatccaac cctagctggt ctcattgccc attccacagc aactgcccag tgcctcacct
 15001 ctacagatcaa ccattgaggc aggaatggag acaagatgac cccaagggtt tttcttctcc
 15061 ctagttcaat ggttttatga tacaaactac tgacatacgt ttttcaagtt attttctcct
 15121 tcttctagga aatcccttct gagtgatgtc acatcttggc aggggtggag gagagcctgg
 15181 ttgcccaggg atttgtcctt ggggacatct catccatcaa gttgcacact cactggcatc
 15241 tttgctatgg ggacattcca atttgcaatt tcaaggaacac tctgaattcc aagtagaatt

FIG. 12 (cont.)

15301 gatttccctt cttctgtcat ctaccttttc tcttcatttt cccattttta ttacccttct
 15361 ttccattttct ctctccagtc ttccacctgg aagccctctc tggctaagga caggcagggtg
 15421 cccctctctc catcagagga cacctgtact ggagagcaac acaggatggt ctctgccatg
 15481 aactggaggc caggaatctc ctactgaaa attacagtat ggtaactttg caaatggtgg
 15541 ttgtttcttc caagactcca gccctgattg cgcaaaactg aaaggcatgt gaagggaagg
 15601 aagaggaaga gtgcaaaaca ttgaagagag agctgagtga gctgaagagt gaggatatga
 15661 gtagcccca cccaaacctg gagatgggga gaaacctaca gaatactagc cagagctcct
 15721 ccttgtcttg gcagcctact agggacctgg ggaagcaaaa acgaaagctg ggcaacatgc
 15781 ctgctttaga atgttttcct tctacttaca catcttcac aggtctcaga atctttcctt
 15841 cctctcatcc ttttctccta tctacatata tatcagagta tccactgttt attcaacaac
 15901 tactacttga tggctcagaca caaacaacaa agctagggtgc taattaataa agatacaggt
 15961 tttggccggg tgcggtggct cagcctgtta atcccagcac tttgggaggc cgaggcgggc
 16021 gaatcacgag gtcaggagtt caagaccagc ctggccaaca tggtgaaacc ccatctctac
 16081 taaaaataca aacaattaac tgagcatagt ggtgggcacc tataatacca gctactccgg
 16141 aggtgaggc aggagaatcg cttgaaccca ggaggcagag gttgcagtga gctgagatcg
 16201 cgccactgca ctctagccgg agtgacagag taagactctg tctcaaaaat aaataaataa
 16261 ataaataaat aaataaatpa ataaataaaa aataataata caagttttca taagcacact
 16321 tctaaccctt tgtctttfat gtatttcctt ccttatccac gcacctgtct cctctactc
 16381 cagcctcatt accccagagg tcagtcctca ggaaaactaa acacaaagaa agagctcagt
 16441 cagaaaggcc atttatttat gtttcaagat gctcactgcc tctttgttt tgtctcctt
 16501 gcaggccttc tctcttaggc ctcttctcct gggggtatgg atcctgggg gagattgatc
 16561 acctccatgc ttccattcct ccccagccat agtggggaca tcatgagaga agccaagcca
 16621 ctggcccagg atcaccgggc atttatggtg gctgctctgg cacaggctct tgcctttata
 16681 gcccctccag tgatccataa ggccctcttt cccccaaag gagaggtcac agatagggca
 16741 aaggtagctc ttctgcttcc agtgggtctg ctggtgctg accagcctgg aaaatgagct
 16801 gaaagacttg ctgcaatgga agcagtagtt gggcggtct gtgaggtggc ccttctggtg
 16861 tctggagaga taggatttct tgctaaaagt caaagaacaa tgggggcaac agaagacatt
 16921 gagtcttgag ggcttcaact gatgagagtt ggatctggca tctgacaga gggttccagt
 16981 gatgggtgcc tgggtcctgg tcacagggtc ttggttctta agtacagatg cctggttctg
 17041 ggccatagga cctcagttc taaatatggg ttctggggac ctggccactg gtgcatggtt
 17101 cacatccaaa agccctgga tggacctctg gcttctggcg atgggtgtct ggaattcagc
 17161 ctgggtgctt ggaatcctca aagtacactc ctggtttcca tccactggct cctggttttg
 17221 gtgtatcttc tgggtggcgt tgagctcaga ctggtcccgg aagctcttcc cacacacaga
 17281 gcatgaatgg ggccggtaac ccagatggac gcggcggtga cgacttagtc cagaagcatc
 17341 acagtaggtc ttgtcacaga gcgtgcaaca gaaggcctc tccccagat gcatgcgtct
 17401 gtgatactg agggacttgg ggctccgaaa caacttccca cactgactgc agctgttagt
 17461 cagcttggga ttgtgaacaa actggtggct atagaggtag gagcgctgc tgaacattt
 17521 ggcacagggt tagcaaaa

FIG. 12 (cont.)

1 tttgtatgtc attgcaggat tcatgctttc cagtgtgtca tctatggaac tgcctctttc
61 ttcttccttt atggggccct cctgctggct gagggcttct acaccaccgg cgctgtcagg
121 cagatctttg gcgactacaa gaccaccatc tgcggcaagg gcctgagcgc aacggtaaca
181 gggggccaga aggggagggg ttacagaggc caacatcaag ctcatcttt ggagcgggtg
241 tgtcattgtt tgggaaaatg gctaggacat cccgacaagg tgatcatcct caggattttg
301 tggcaataac aaggggtggg gggacaa

FIG. 13

1 ctgtatcagt gctcctcgtc gctcactgt acttcacgga agagacttgg ttgactggcc
 61 acttgagcgc gaatcaggag acattcccaa ctacagagaga ctgagcccta gctcgccac
 121 ttgctggaca agatgatatt ccttaccacc ctgcctctgt tttggataat gatttcagct
 181 tctcgagggg ggcactgggg tgcttgatg ccctcgccca tctcagcctt cgagggcacg
 241 tgtgtctcca tcccctgccg ttccgacttc ccgatgagc tcagaccggc tgtggtacat
 301 ggcgtctggt atttcaacag tccctacccc aagaactacc cgccagtggc cttcaagtcc
 361 cgcacacaag tgggtccacga gagcttccag ggccgtagcc gcctgttggg agacctgggc
 421 ctacgaaact gcaccctgct tctcagcacg ctgagccctg agctgggagg gaaatactat
 481 ttccgaggtg acctgggcgg ctacaaccag tacaccttct cgagcacagc cgtcctggac
 541 atcatcaaca cccccaacat cgtgggtgccc ccagaagtgg tggcaggaac ggaagtagag
 601 gtcagctgca tgggtgccga caactgccc gagctgcgcc ctgagctgag ctggctgggc
 661 cagcaggggc taggggagcc cactgttctg ggtcggctgc gggaggtaga aggcactggg
 721 gtgcaggtgt cactgctaca cttcgtgcct actagagagg ccaacggcca ccgtctgggc
 781 tgtcaggtct ccttcccaa caccaccttg cagttcgagg gttacgccag tctggacgtc
 841 aagtaccccc cgggtgattgt ggagatgaat tctctgtgg aggccattga gggctccac
 901 gtcagcctgc tctgtggggc tgacagcaac ccgccaccgc tgctgacttg gatgctggat
 961 gggatggtgt tgaggaggc agttgctgag agcctgtacc tggatctgga ggaggtgacc
 1021 ccagcagagg acggcatcta tgcttgccct gcagagaatg cctatggcca ggacaaccgc
 1081 acgggtggagc tgagcgtcat gtatgcacct tggaaagcca cagtgaatgg gacggtgggtg
 1141 gcggtagagg gggagacagt ctccatcctg tggtccacac agagcaaccc ggaccctatt
 1201 ctaccatctt tcaaggagaa gcagatcctg gccacggtca tctatgagag tcagctgcag
 1261 ctggaactcc ctgcagtgc gcccaggagc gatggggagt actggtgtgt agctgagaac
 1321 cagtatggcc agagagccac cgccttcaac ctgtctgtgg agtttgcctt cataatcctt
 1381 ctggaatcgc actgtgcagc ggccagagac accgtgcagt gcctgtgtgt ggtaaaatcc
 1441 aacccggaac cctcctgggc ctttgagctg ccttcccgc acgtgactgt gaacgagaca
 1501 gagagggagt ttgtgtactc agagcgcagc ggccctcctg tcaccagcat cctcacgctc
 1561 cggggtcagg cccaagcccc accccgcgtc atttgtacct ccaggaacct ctacggcacc
 1621 cagagcctcg agctgccttt ccagggagca caccgactga tgtgggcca aatcggccct
 1681 gtgggtgctg tggtcgcctt tgccatcctg attgccattg tctgctacat caccagaca
 1741 agaagaaaaa agaacgtcac agagagcccc agcttctcag cgggagacaa ccctcatgtc
 1801 ctgtacagcc ccgaattccg aatctctgga gcacctgata agtatgagag tgagaagcgc
 1861 ctgggggtccg agaggaggct gctgggcctt aggggggaac cccagaact ggacctcagt
 1921 tattccact cagacctggg gaaacgacct accaaggaca gctacacctt gacagaggag
 1981 ctggctgagt acgcagaaat ccgagtcaag tga

FIG. 14

1 masqkrpsqr hgs kylatas tmdharhgfl prhrdtgild sigrffggdr gapkrsgkd
61 shhpartahy gslp qkshgr tqdenpvvhf fknivtprtp ppsqgkgrgl slsrfswgae
121 gqrp gfgygg rasdyksahk gfkgvdaqgt lskifklggr dsrsgspmar r

FIG. 15

FIG. 15

1 mglleccarc lvgapfaslv atglcffgva lfcgcgheal tgtekliety fsknyqdyey
61 linvihafqy viygtasfff lygalllaeg fyttgavrqi fgdyktticg kglsatvtgg
121 qkgrgsrgqh qahslervch clgkwlghpd kityaltvvw llvfacsavp vyiynfntwtt
181 cqsiafpskt sasigslcad armygvlpwn afpgkvcgsn llsicktaef qmtfhlfiaa
241 fvgaaatlvs lltfmiaaty nfavklmgrp gtkf

FIG. 16

```
1 maslsrpslp sclcsfllll llqvsssyag qfrvigprhp iralvgdeve lpcrispgkn
61 atgmevgwyr ppfsrvvhly rngkdqgdgq apeyrgrtel lkdaigegkv tlrirnvrfs
121 deggftcfr dhsyqeeaaam elkvedpfyw vspgvlvlla vlpvlllqit lglvflclqy
181 rlrghklraei enlhrtfdph flrvpcwkit lfvivpvlgp lvaliicynw lhrrlagqfl
241 eelrnpf
```

FIG. 17

**DECLARATION
AND POWER OF ATTORNEY**

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below at 201 et seq. underneath my name.

I believe I am the original, first and sole inventor if only one name is listed at 201 below, or an original, first and joint inventor if plural names are listed at 201 et seq. below, of the subject matter which is claimed and for which a patent is sought on the invention entitled

ACTIVATED T-CELLS, NERVOUS SYSTEM-SPECIFIC ANTIGENS AND THEIR USES

and for which a patent application:

- ☒ is attached hereto *(if applicable)*
☐ was filed in the United States on _____ as Application No. _____ with Preliminary Amendment filed on _____ *(if applicable)*
☐ was filed as PCT international Application No. _____ on _____ and was amended under PCT Article 19 on _____ *(if applicable)*

I hereby state that I have reviewed and understand the contents of the above identified application, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119(a)-(d) of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

EARLIEST FOREIGN APPLICATION(S), IF ANY, FILED PRIOR TO THE FILING DATE OF THE APPLICATION			
APPLICATION NUMBER	COUNTRY	DATE OF FILING (day, month, year)	PRIORITY CLAIMED
IL 124550	Israel	May 19, 1998	YES <input checked="" type="checkbox"/> NO <input type="checkbox"/>
			YES <input type="checkbox"/> NO <input type="checkbox"/>

I hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional application(s) listed below.

APPLICATION NUMBER	FILING DATE

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code §112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

APPLICATION SERIAL NO.	FILING DATE	STATUS		
		PATENTED	PENDING	ABANDONED
PCT/US98/14715	July 21, 1998		X	

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

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DATE	DATE	DATE